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Small-scale spatial variability of phenoxy acid mineralization potentials in transition zones with a multidisciplinary approach

Meriç Batıoğlu-Pazarbaşı

DTU Environment
Department of Environmental Engineering

PhD Thesis
March 2013

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The synopsis part of this thesis is available as a pdf-file for download from the DTU research database ORBIT: http://www.orbit.dtu.dk

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PREFACE

This PhD thesis is based on the research conducted at Geological Survey of Denmark and Greenland (GEUS) between the period of May 2009–October 2012 with Prof. Hans-Jørgen Albrecthsen as university advisor, Prof. Jens Aamand as principle advisor and Dr. Sebastian Reinhold Sørensen as co-advisor. The PhD project was funded by Research Training for Good European Groundwater Resources, Support for training and career development of researchers, European Union 7th Framework Programme (Marie Curie Actions), Grant Agreement Number 212683. The PhD thesis comprises a synopsis of the studies addressing the small-scale spatial distributions of phenoxy acid-mineralization potentials depending on *in situ* herbicide exposure and the bacterial ecology in two important transition zones (1) unsaturated–saturated zone interface and (2) groundwater–surface water interface, and four scientific papers as follows:

I. Batıoğlu-Pazarbaşı M, Bælum J, Johnsen AR, Sørensen SR, Albrechtsen H-J & Aamand, J (2012) Centimetre-scale vertical variability of phenoxy acid herbicide mineralization potential in aquifer sediment relates to the abundance of *tfdA* genes. *FEMS Microbiology Ecology* 80: 331–341.

II. Batıoğlu-Pazarbaşı M, Sørensen SR & Aamand, J (2012) Herbicide concentration effect on centimetre-scale spatial distribution of MCPA mineralization potential along the transition zone between unsaturated and saturated zones. Manuscript.

III. Batıoğlu-Pazarbaşı, M, Milosevic, N, Malaguerra, F, Binning, PJ, Albrechtsen, H-J, Bjerg, PL & Aamand, J (2013) Discharge of landfill leachate to streambed sediments impacts the mineralization potential of phenoxy acid herbicides depending on the initial abundance of *tfdA* gene classes. *Environmental Pollution* DOI: 10.1016/j.envpol.2013.01.050.

IV. Batıoğlu-Pazarbaşı M, Bælum J, Pilloni G, Larentis M, Jacobsen CS, Lueders T, Hansen LH & Aamand J (2012) Bacterial adaptive response to changing herbicide discharge rates in the streambed sediments impacted by a landfill. Manuscript.

In this online version of the thesis, the papers are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from: DTU Environment, Technical University of Denmark, Miljoevej, Building 113, 2800 Kgs. Lyngby, Denmark, reception@env.dtu.dk.

ACKNOWLEDGEMENTS

My PhD journey started three years ago after getting selected as a Marie Curie PhD fellow. It was the most challenging but also the most educative experience of my life. Doing PhD is a bittersweet way of reaching the success, but it is really worth it.

I first want to thank my principal advisor, Prof. Jens Aamand. Jens has always encouraged me to write my own research plans as subprojects of my PhD study. This allowed me to work independently and creatively. However, his door was always open whenever I had a question and most importantly, he kept his positive attitude during whole my PhD study. Besides, his recommendations were very helpful to improve my scientific writing skills. I thank my co-advisor, Dr. Sebastian R. Sørensen for his comments on writing my first and second manuscripts. I also thank my university advisor, Prof. Hans-Jørgen Albrechtsen for all scientific discussions during my research, his helpful recommendations throughout my thesis and his revisions on my danish summary.

I thank Dr. Anders R. Johnsen for his collaboration and his valuable comments on my first manuscript. Thanks to Prof. Carsten S. Jacobsen for letting me use his molecular lab facilities and feedbacks on my fourth manuscript. I also thank all of our technicians, especially Jens Bisgaard and Spire M. Kiersgaard for their amazing help in core slicing. I thank Prof. Poul L. Bjerg and Prof. Phillip J. Binning for the great collaboration and their thorough feedbacks on my third manuscript and thanks to Poul again for allowing me to use samples from Risby landfill site for my fourth project. Thanks also to all co-authors from DTU in my third manuscript.

My special thanks to Dr. Jacob Bælum for his extremely helpful attitude, valuable guidance and encouragement. During my PhD studies, Jacob answered all of my questions patiently even in his postdoc period in the US via e-mails. It was a pleasure to collaborate with him for my first and fourth manuscripts. I also would like to thank him helping me to enter the world of bioinformatics by showing how to deal with metagenomic data. Jacob has great supervision skills, he never drove the car for me but showed well how to do it and supported whenever I needed. His approach gave me a great opportunity to improve my scientific skills in lab, genomic/metagenomic data handling and scientific writing. I also would like to thank Jacob for translating my thesis summary to Danish in his busy (!) period of paternity leave :)

Thanks also to Assoc. Prof. Lars H. Hansen for his close guidance in his lab during my pyrosequencing study and valuable comments in my fourth manuscript. I thank Karin Vestberg for her technical assistance during my stay in Lars' lab. I also thank my husband, Ismail Pazarbaşı for helping me to install Phyton script library to run latest version of QIIME natively in my MacBook Pro. I also appreciate his help to get familiar with Phyton scripting language. Thanks also to all inspiring QIIME developers and their quick help in QIIME forum.

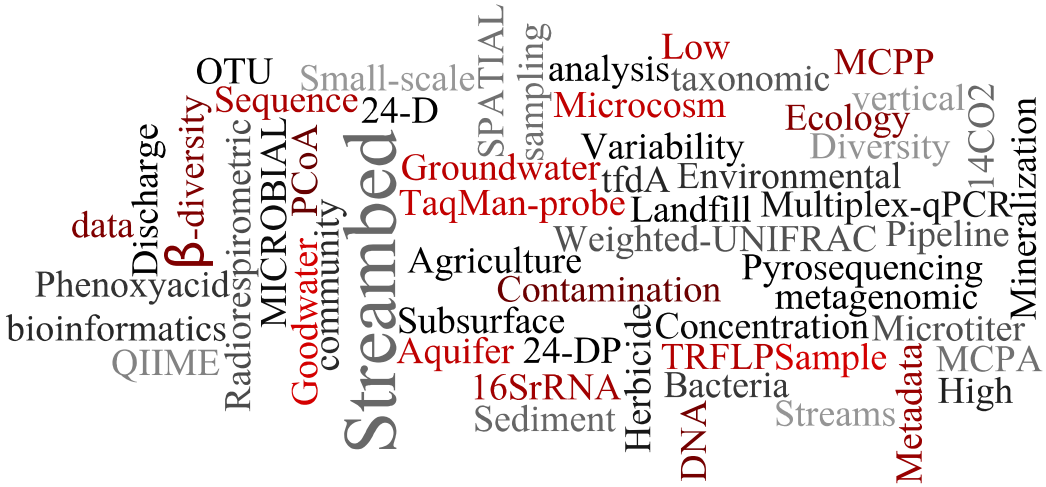
It was a privilege to be involved in Goodwater Initial Training Network. I had the opportunity to interact with other research institutes and universities in Germany and Belgium during workshops, summer- and winter-schools. Furthermore, I worked in Dr. Tillmann Lueders' lab in Germany. I thank him and his group for their help in T-RFLP and PCA analysis.

I would like to thank my dear parents, Necla and Muharrem for their great support during all my education life. Due to their young spirits I always feel like I have two best friends rather than parents, thanks to their love for 30 years.

Lastly, I thank my beloved husband again for letting me chase my career and live in another country for three years. I also thank him giving me both moral support with his cheerful personality and technical support with his computer programming expertise.

Copenhagen, October 2012
Meriç Batıoğlu-Pazarbaşı

“The important thing is not to stop questioning. Curiosity has its own reason for existing”, - Albert Einstein.



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SUMMARY

The phenoxy acid group of herbicides is widely used to control broadleaf weeds, and it contaminates groundwater and surface water by leaching from agricultural soil or landfills. Due to the distinct vertical and horizontal gradients in nutrients and hydrologic exchange in transition zones, the challenge of small-scale spatial variability occurs in terms of microbiological and physicochemical properties. We used genomic- and metagenomic-based approaches to reveal the effects of long-term phenoxy acid *in situ* exposure on the small-scale spatial variability of herbicide mineralization potentials in two transition zones, (1) the interfaces of unsaturated and saturated zones and (2) groundwater and surface water.

Small-scale spatial variability of phenoxy acids was previously shown in topsoil; however, such small-scale studies are scarce in subsurface environments. We therefore studied the factors shaping the centimetre-scale vertical variability of phenoxy acid degradation in the interface of unsaturated and saturated zones. We demonstrated more even centimetre-scale vertical distribution of MCPA mineralization potential than that of 2,4-D at the concentration of 1 mg kg^{-1}, and the mineralization activity was linked to the abundance of *tfdA* genes involved in phenoxy acid degradation depending on the MCPA leaching from the overlying agricultural soil expressed as long-term *in situ* MCPA exposure. Knowledge on the small-scale variability of mineralization potential is very important to predict the fate and transport of herbicides in aquifer sediments.

Due to a more widespread mineralization potential, we used MCPA as a model herbicide to determine the effects of herbicide concentration and additional carbon source on the centimetre-scale vertical distribution of mineralization potential along the unsaturated-saturated zone interface. We determined higher MCPA degradation activity at environmentally relevant concentrations (~15 µg kg^{-1}) compared to high concentrations (10 mg kg^{-1}) indicating the greater number of bacteria adapted to low MCPA concentration. We further showed that the addition of external carbon sources such as soil extract and benzoic acid could stimulate cometabolic degradation in the ring structure rather than the carboxyl group of MCPA. These findings might be important to develop bioremediation strategies for slightly contaminated subsurface environments.

Old landfills are major sources of multiple contaminants detected in groundwater and surface water. In this PhD thesis, the mineralization potentials of phenoxy acids in landfill-impacted groundwater-surface water interface were determined.

The phenoxy acids were mineralized to different extents in response to changing herbicide mass discharges determined by multiplying the specific groundwater discharge with herbicide concentrations. The differences in mineralization potentials were linked to the initial abundance of *tfdA* gene classes. The Monod-based kinetic model confirmed the role of initial abundance of *tfdA* gene classes in the different mineralization potentials of discharge zones. Understanding of the natural attenuation potential of groundwater-surface water transition zones is important for stream water protection.

In landfill-impacted groundwater-surface water interface, we further analyzed bacterial communities with a combined genomic-metagenomic approach. Principal component analysis disclosed the clustering of bacterial diversity determined by terminal restriction length polymorphism and 16S rRNA amplicon pyrosequencing according to discharge zones. This comprehensive analysis indicated that specific phenoxy acid degraders proliferated in the streambed sediments in response to changing discharge rates of landfill-phenoxy acids; and the bacterial communities differentiated after mineralization at environmentally relevant and higher concentrations. Our results suggested that the clustering of bacterial communities depending on the landfill impact could be used as microbial ecological indicators for more advanced risk assessment analysis.

In conclusion, this PhD thesis provided information about the mineralization potential of phenoxy acids depending on the long-term *in situ* herbicide exposure and bacterial ecology. The findings based on our multidisciplinary approach including engineering, genomics/metagenomics, bioinformatics and multivariate statistics could contribute to develop novel bioremediation strategies in the future.

DANSK SAMMENFATNING

Gruppen af phenoxysyreherbicider bruges i vid udstrækning til at bekæmpe to-kimbladet ukrudt, og denne anvendelse har ført til grundvands- og overfladevandsforurening som følge af udvaskning fra landbrugsjord eller gamle lossepladser. Sådanne forureninger er undersøgt med hensyn til rumlig variation som følge af vertikale og horisontale gradienter fx i næringsstofkoncentration og hydrauliske forhold i overgangszoner. Denne variation i fysisk-kemiske forhold kan føre til mikrobiel variation på både lille og større skala. Ved hjælp af genom- og metagenombaserede tilgange undersøgte vi, hvorledes langvarig in situ eksponering med phenoxysyrer påvirkede den rumlige småskala variation af mineraliseringspotentialet for herbicider i to overgangszoner: grænsefladerne mellem (1) umættede og mættede zoner og (2) grundvand og overfladevand.

Der er tidligere påvist rumlig småskala variation af phenoxysyrenedbrydning i topjord, men der er ikke udført tilsvarende undersøgelser i dybere lag. Vi har derfor på centimeter-skala undersøgt faktorer med betydning for den vertikale variation af phenoxysyrenedbrydning i grænsefladen mellem umættede og mættede zone. Ved en koncentration på 1 mg kg^{-1} påviste vi mindre vertikal variation i potentialet for mineralisering af MCPA end af 2,4-D. Endvidere var der øget forekomst af *tfdA* gener, der er involveret i phenoxy syrenedbrydning, afhængigt af udvaskningsgraden af MCPA fra overliggende landbrugsjord. Viden om småskala-variation i mineraliseringspotentialet er meget vigtig for at kunne forudsige skæbne og transport af herbicider i grundvandssedimenter.

På grund af MCPA's højere mineraliseringspotentiale, anvendte vi MCPA som modelherbicid til at undersøge, hvorledes herbicidkoncentration og ekstra kulstofkilder påvirkede den vertikale fordeling på centimeter-skala af mineraliseringspotentialet langs grænsefladen mellem den umættede og mættede zone. MCPA-nedbrydningen var mere jævnt fordelt ved miljømæssigt relevante koncentrationer (~15 µg kg^{-1}) end ved højere koncentrationer (10 mg kg^{-1}), hvilket indikerer at de specifikke nedbrydere var tilpasset en lav MCPA-koncentration. Desuden stimulerede tilsætning af eksterne kulstofkilder, som jordekstrakt og benzoesyre co-metabolisk nedbrydning af ringstrukturen i forhold til carboxylgruppen i MCPA. Vores resultater tyder på, at de bakterier, der er i stand til at mineralisere en lav MCPA koncentration bør tages i betragtning til udvikling af bioremedieringsstrategier for svagt forurenede miljøer i undergrunden.

Gamle lossepladser er ofte kilde til forureninger af grundvand og overfladevand. I denne ph.d.-afhandling blev mineraliseringspotentialet af phenoxysyrer undersøgt i grænsefladen imellem mættet og umættet zone i sediment i bunden af et vandløb, der er påvirket udsivende grundvand med lossepladsperkolat. De forskellige phenoxysyrer blev mineraliseret i forskelligt omfang som følge af udsivning af herbicid. Forskellene i mineraliseringspotentiale var knyttet til den initielle forekomst af *tfdA* gen i sedimentet. En Monod-baseret model bekræftede betydningen af den initielle forekomst af *tfdA* gener for mineraliseringenspotentialet i forskellige udledningszoner. Forståelsen af det naturlige potentiale i den mættede/umættede overgangszone er vigtig for beskyttelsen af vores overfladevand.

De bakterielle samfund i den mættede/umættede zone i lossepladspåvirket sediment er analyseret ved en samlet genom-metagenom tilgang. Principal komponent analyse af T-RFLP data og 16S rRNA gene amplikon pyrosekventering viste en gruppering af bakteriel diversitet i relation til udledningszonerne. Denne omfattende analyse viste en tilvækst i specifikke phenoxysyrenedbrydere i vandløbssedimentet i udledningszonen som følge af ændringer i udledningen af lossepladsrelaterede phenoxysyrer. De bakterielle samfund differentierede efter mineralisering ved miljømæssigt relevante og højere koncentrationen af phenoxysyrer. Vores resultater antyder, at grupperingen af bakterielle samfund afhængigt af påvirkningen fra lossepladsen vil kunne anvendes som mikrobiel indikator i mere avancerede risikovurderings analyser.

Denne ph.d.-afhandling bidrager med ny viden om potentialer for phenoxysyrenedbrydning efter langvarig eksponering og om bakteriel økologi forbundet hermed. Resultaterne er baseret på vores tværfaglige tilgang, herunder feltteknik, genomic/metagenomics, bioinformatik og multivariat dataanalyse og kan bidrage til at udvikle nye bioremedieringsstrategier i fremtiden.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 BACKGROUND AND RATIONALE

Transition zones are dynamic ecotones characterized by steep chemical and biological gradients; and play a critical role in biofiltering of pollutants to protect groundwater and surface water systems. However, transition zones are complex environments due to their spatially variable hydrogeological, geochemical and microbiological properties. The multidisciplinary efforts are therefore needed to understand the variability issues in such environments to predict the fate and transport of contaminants and to protect the drinking water quality. The interfaces of unsaturated and saturated zones (i.e. the surrounding region of groundwater table) and of groundwater and surface water (i.e. hyporheic zone) are important transition zones.

Phenoxy acids mimicking natural plant growth hormone (i.e. indoleacetic acid) are commonly used herbicides worldwide to control the broadleaf weeds. Phenoxy acid herbicides are often found as contaminants in groundwater environments originating not only from agricultural practice (Larsen *et al.*, 2000), but also as leachate from landfills (Gintautas *et al.*, 1992; Harrison *et al.*, 1998; Tuxen *et al.*, 2003; Haarstad & Maehlum, 2008). Due to low sorption and high water solubility, phenoxy acids have a high potential to leach into ground and surface water systems (Tuxen *et al.*, 2000*)*. Despite the many studies addressing the small-scale spatial variability of phenoxy acid mineralization potentials in soil (Gonod *et al.*, 2003, 2006a; Rodriguez-Cruz *et al.*, 2009; Sjøholm *et al.*, 2010), the knowledge of small-scale variability is limited in subsurface environments. Even though Janniche *et al.* (2011) has recently reported the small-scale vertical variability of sorption and herbicide degradation in the sediment samples from an unsaturated limestone aquifer and a sandy aquifer, comprehensive variability studies are not available regarding to the spatial distributions of phenoxy acid mineralization potentials, the abundance of functional genes and the bacterial community structures in transition zones.

1.2 PHD THESIS OBJECTIVES

This thesis includes hypotheses about the factors controlling the small-scale spatial variability of mineralization potentials such as long-term *in situ* phenoxy acid herbicide exposure and bacterial ecology in two transition zones of Denmark, (1) the interface of unsaturated and saturated zones in Fladerne Creek and (2) the interface of groundwater and surface water along the Risby stream

close to an inactive landfill,

(i) to test whether vertical variation in mineralization potential of phenoxy acids exists in the oligotrophic (i.e. nutrient-poor) saturated zone at centimetre scale and to correlate it with microbiological and physicochemical characteristics (Batıoğlu-Pazarbaşı *et al.*, I),

(ii) to test whether herbicide concentration and additional external carbon sources impact centimetre-scale vertical variation in metabolic /cometabolic mineralization potentials of phenoxy acids in the surrounding region of a groundwater table (i.e., the oligotrophic interface of unsaturated and saturated zones) (Batıoğlu-Pazarbaşı *et al.*, II),

(iii) to test whether the difference in mineralization potential of phenoxy acids exists in the streambed sediments from three zones receiving landfill impacted-groundwater at changing discharge rates and to correlate it with microbiological and physicochemical characteristics (Batıoğlu-Pazarbaşı *et al.*, III)

(iv) to test whether changing herbicide mass discharges impact bacterial community structures in the same streambed sediments used in the previous study (Batıoğlu-Pazarbaşı *et al.*, IV).

1.3 PHD THESIS OUTLINE

In Chapter 2, transitional environments including the interfaces of unsaturated and saturated zones, and of groundwater and surface water were described. In Chapter 3, the phenomenon of spatial variability was mentioned in all of the aspects including hydrogeology, geochemistry and microbiology. The sampling approaches taking the small-scale spatial variability into account were also mentioned with the relevant examples of literature. In Chapter 4, phenoxy acid herbicides were mentioned in detail including contamination source, degradation pathway and the modelling of mineralization kinetics. In Chapter 5, bacterial ecology in contaminated environments including soil, groundwater and surface water systems were mentioned. Specific degraders (oligotrophs and copiotrophs), adaptation of bacteria to long-term herbicide exposure at low concentrations and genomic/metagenomic-based approaches in environmental microbial ecology were particularly mentioned. Chapter 6 highlights key findings and conclusions, and Chapter 7 gives the future directions based on the conclusions.

2 TRANSITION ZONES (ECOTONES)

2.1 UNSATURATED–SATURATED INTERFACE

The transitional zones between different adjacent ecosystems are called as ecotones in ecology, and characterized by a high biodiversity and biological activity. Goldscheider *et al.* (2006) reported that the ecotone concept could be applied to subsurface environments. For instance, the most important ecotone in an aquifer is the groundwater table with a capillary fringe and the region immediately below the water table (i.e. unsaturated–saturated interface). This transition zone is a highly dynamic environment with its hydrological, geochemical and microbiological characteristics (Silliman *et al*., 2002; Berkowitz *et al.*, 2004). Earlier studies also focused on this transition zone, and showed its spatial variability in terms of water content, gas-filled porosity and bacterial activity (Ronen *et al*., 1997; Affek *et al*., 1998; Ronen *et al*., 2000).

In Batıoğlu-Pazarbaşı *et al.*, I, the highest mineralization potential of phenoxy acids (2,4-D and MCPA) was observed in the region immediately below the groundwater table. However, the mineralization potentials displayed variability in the deeper regions of the aquifer. Furthermore, MCPA was chosen as a model compound due to its more even distribution of mineralization potential in the saturated zone than that of 2,4-D in Batıoğlu-Pazarbaşı *et al.*, II indicating that not only the region below the groundwater table but also the region above the groundwater table act as biofilter against contaminants.

The groundwater table fluctuations cause the spatial distribution of contaminants and degradation potentials. Sinke *et al.* (1998) examined the effect of water-table fluctuations on redox conditions and the transport of dissolved toluene and 4-nitrobenzoate within a sand column and observed marked differences in contaminant transport and redox conditions within the column after repeated fluctuations of the water table. Dobson *et al.* (2007) determined that water-table fluctuation led to enhanced biodegradation activity and the dissolutions of hexadecane, toluene, ethylbenzene and 2-methylnapthalene within an aquifer. The groundwater fluctuations due to season differences were also observed in the sandy aquifer of Fladerne Creek. The groundwater table was at 2.50 meter below ground surface (m b.g.s.) when a sediment core was collected from the saturated zone on June (Batıoğlu-Pazarbaşı *et al.*, I). However, the groundwater table increased to 1.88 m b.g.s. when two sediment cores were collected from both unsaturated and saturated zones on October (Batıoğlu-Pazarbaşı *et al.*, II). Higher

mineralization potential of phenoxy acids observed in the surrounding region of groundwater table might also be attributed to the fluctuation of groundwater table in Fladerne creek as illustrated in Figure 1. The spatial variability of mineralization potentials of phenoxy acids in aquifers due to preferential flow paths or groundwater table fluctuations was discussed in Chapter 3.

2.2 GROUNDWATER–SURFACE WATER (GW–SW) INTERFACE

The GW-SW interface is a dynamic transitional zone providing a range of ecosystem services and controls the movement of contaminated water between surface and subsurface environments (Smith *et al.*, 2009). The understanding of interactions between groundwater and surface water (GW–SW) is necessary for the effective management of water resources (Sophocleous, 2002). However, these interfaces are complex and spatially variable in terms of hydrology, geochemistry and microbiology.

The spatial and temporal varability of natural attenuation processes in GW–SW transition zones are controlled by factors such as the sediment properties including thickness, geochemistry, mean grain size and permeability, and the hydraulic gradients which drive exchange between surface and subsurface environments (Smith and Lerner, 2008; Smith *et al.*, 2009). Temperature profiling and flux estimates using seepage meters or head measurements were used to determine the spatial and temporal variability of water movement across GW–SW transition zones (White *et al.* 1987; Becker *et al.* 2004; Conant, 2004). The spatial variability of groundwater discharges and herbicide concentrations resulting in herbicide mass discharges and its effects on the mineralization potential of phenoxy acids was discussed in Chapter 3.

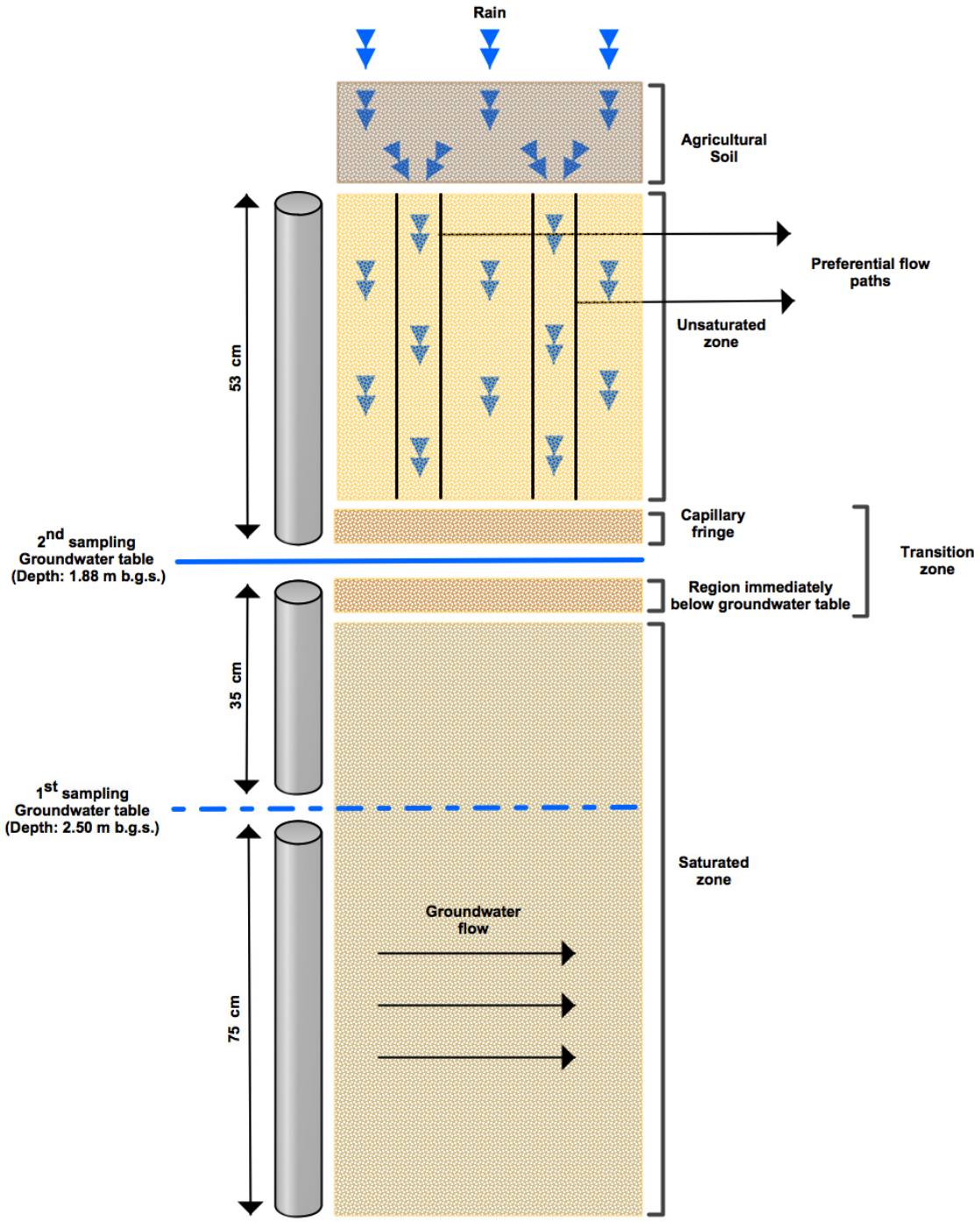

Figure 1. Schematic illustration of water flow via preferential flow paths in an unsaturated zone (Fladerne Creek, Denmark). The locations of sediment cores for the first and second studies of thesis were also demonstrated. The drawing of preferential flow paths in the unsaturated zone was taken from Kim *et al.*, 2005 and adapted to situation in Fladerne Creek by adding specific layers (i.e. capillary fringe, groundwater table, saturated zone), direction of groundwater flow and cores.

3 SMALL-SCALE SPATIAL VARIABILITY AND SAMPLING APPROACHES

Spatial variability can be defined as the interaction of physical, chemical, and biological variables influencing the abundance, diversity, and activity of microorganisms at many different spatial scales (Franklin and Mills, 2007). In this chapter, the factors shaping spatial variability in soil and subsurface environments with hydrogeological, geochemical and microbiological aspects and small-scale sampling approaches were given below:

3.1 SMALL-SCALE SPATIAL VARIABILITY

3.1.1 HYDROLOGY

The term preferential flow refers to the rapid, nonuniform transport of solutes and water through preferred pathways (Stagnitti *et al.*, 1994). The dissolved pollutants can be transported through the unsaturated zone along preferential pathways (Swensen, 1997). The quantification of preferential flow is important to understand the hydrological spatial variability more accurately in the unsaturated zone. Stumpp *et al.* (2007) developed a method to quantify the heterogeneity of the water flux in the unsaturated zone by applying environmental isotope data and a lumped parameter approach. The authors noted that the preferential flow path has a high water flow velocity (short transit time), which may transport the pollutant rapidly to greater depths and can seriously contaminate the groundwater. The same method also revealed that soils and land use have distinct impacts on the quantity of preferential flow and flow heterogeneities in the unsaturated soil planted with different crops (Stumpp and Maloszewski, 2010). The effect of preferential flow paths on the microbial abundance was also studied (Bundt *et al.*, 2001). The authors reported that preferential flow paths cause greater microbial abundance due to more favorable living conditions in preferential paths than the rest of the soil matrix.

The preferential flow also occurs in the unsaturated zone above the sandy aquifer in Fladerne Creek sampled for two studies in the thesis (Batıoğlu-Pazarbaşı *et al.*, I and II). The infiltration water including possible pollutants passes through vertical canalized fingers after intensive precipitation (Jacobsen *et al.*, 1998). The water flow with the preferential flow paths in the unsaturated zone was illustrated in Figure 1.

The discharge of contaminated groundwater may contribute a contaminant load

into surface water and sediments (Ford *et al.*, 2006). Recently, the spatial variability in groundwater discharges was investigated by using streambed temperatures (Schmidt *et al.*, 2008). Lower contaminant concentrations were determined in the streambed sediments at high groundwater-discharge locations compared to low discharge locations indicating that the groundwater discharge caused the release of contaminants from the streambed to the stream water. However, knowledge about the discharge effect of contaminated groundwater on the geochemistry and microbiology in GW–SW transition zones is still limited; and these issues need to be elucidated (Röling *et al.*, 2001; Lien, 2006).

The two studies of thesis addressed the discharge effect of landfill impacted-groundwater on the phenoxy acid mineralization potentials, abundance of *tfdA* gene classes, and bacterial diversity of streambed sediments (Batıoğlu-Pazarbaşı *et al.*, III and IV). The sampling locations along the Risby Stream were illustrated in Figure 2. The spatial variability in groundwater discharges and herbicide concentrations was also demonstrated to explain discharge zones accurately. The mass discharges of landfill-derived herbicides to the seepage meters (SMs) installed in the discharge zones were both correlated with the mineralization potentials and the initial abundance of *tfdA* gene classes involved in phenoxy acid degradation (Batıoğlu-Pazarbaşı *et al.*, III). The spatial variability of bacterial diversity between discharge zones was also determined with a combined approach including genetic fingerprinting method, T-RFLP (Terminal Restriction Length Polymorphism) and 16S rRNA amplicon pyrosequencing with multivariate statistical tools (Batıoğlu-Pazarbaşı *et al.*, IV).

3.1.2 GEOCHEMISTRY

The efficiency of most transformation processes depends on the presence of steep redox gradients including typically complex patterns of aerobic/anaerobic conditions and the presence of organic matter and microbial activity in the hyporheic zone (i.e. the region just below the streambeds) (Fisher *et al.* 1998). The spatial variations of groundwater discharges and of the concentrations of discharging phenoxy acids were determined in a landfill impacted GW–SW transition zone (Batıoğlu-Pazarbaşı *et al.*, III). The spatial variability in the leachate indicators such as electrical conductivity, chloride and dissolved organic carbon confirmed the differences of landfill impact on GW–SW transition zones.

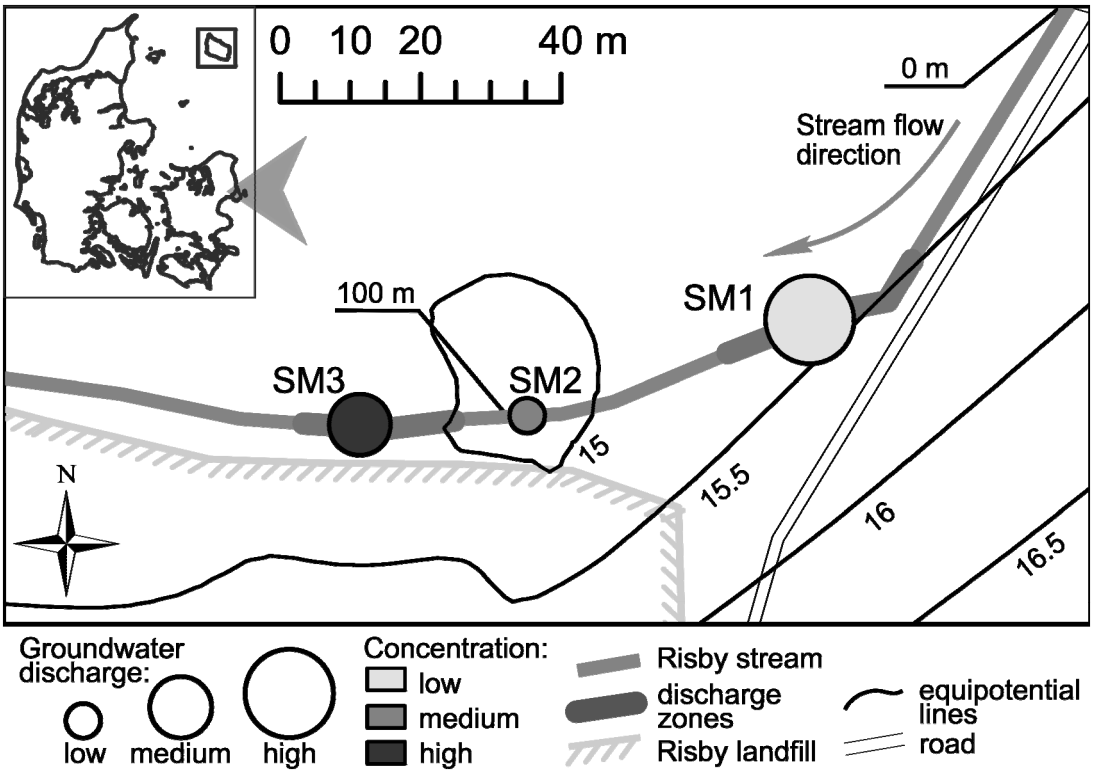

Figure 2. Schematic illustration of sampling along a stream close to Risby landfill. The groundwater was collected from the seepage meters (SM1, SM2 and SM3) installed in three distinct discharge zones. The streambed sediments were collected from the regions near SMs. Sampled discharge zones have different herbicide mass discharges due to variations in either groundwater discharge or herbicide concentration.

3.1.3 MICROBIOLOGY

Microorganisms are not distributed uniformly in the environment, rather their abundance and activity change along environmental gradients (Franklin & Mills, 2003). The spatial variability of mineralization potentials was studied for a number of pesticides including isoproturon (Bending *et al.*, 2001; El Sebai *et al.*, 2007), 2,4-D (Gonod *et al.*, 2003), linuron (Rasmussen *et al.*, 2005), BAM (Sjøholm *et al.*, 2010) and MCPP (Janniche *et al.*, 2011) in soil and groundwater samples. In Batıoğlu-Pazarbaşı *et al.*, I, centimetre-scale spatial variability was observed in the mineralization potentials of phenoxy acids (2,4-D, MCPA, MCPP) and correlated to the abundance of *tfdA* gene classes in the aquifer sediment collected from the saturated zone. However, in Batıoğlu-Pazarbaşı *et al.*, II, MCPA was chosen as a model compound due to its more favorable substrate characteristics when compared to 2,4-D. The effect of MCPA concentration and additional carbon sources on the centimetre-scale spatial distribution of mineralization potential was studied in the surrounding region of

groundwater table. In this study, the higher mineralization potential of MCPA herbicide was observed at environmentally relevant concentration when compared to higher concentration indicating more abundant bacteria adapted to low concentration in an oligotrophic aquifer.

3.2 Small-scale Sampling Approaches

The small-scale sampling approaches are necessary to understand the spatial variations in hydrogeology, geochemistry and microbial communities (Lehman 2007; Franklin and Mills, 2007; Weiss and Cozzarelli, 2008). Sampling strategies should be developed to encompass the spatial variability of the whole area of interest and to obtain representative samples (van Elsas & Smalla, 1997). In the number of studies focusing on the spatial variability of pesticide mineralization potentials, small-scale sampling strategies were developed. For instance, Gonod *et al*. (2003) used two small-scale sampling procedures: (1) the separation of aggregates from the soil and (2) the dissection of soil cores into millimetre range cubes. These sampling approaches enabled the researchers to measure mineralization rates on very small soil samples and to demonstrate a large variability of 2,4-D mineralization potential in soil aggregates or dissected soil cubes of millimetre scale. Gonod *et al*. (2006a) performed two types of 2,4-D mineralization experiments on a field scale at the decametre to the metre scale and at a metre to decimetre scale in the first experiment; and on a microhabitat scale at metre to millimetre scales in the second experiment. The researchers combined geostatistical analysis with this small-scale sampling approach and concluded that 2,4-D mineralization was spatially structured only at the microhabitat scale.

The centimetre-scale spatial variability of phenoxy acid herbicide mineralization potential was also determined in an oligotrophic aquifer with a small-scale sampling strategy as described in Batıoğlu-Pazarbaşı *et al.*, I and II. Briefly, cores were sliced horizontally; and small-scale samples were collected from each slice via a circular grid. The sediment samples were transferred to 96-well microplates to measure herbicide mineralization potentials. The details of sampling approach for Batıoğlu-Pazarbaşı *et al.*, II were given as an example in Figure 3.

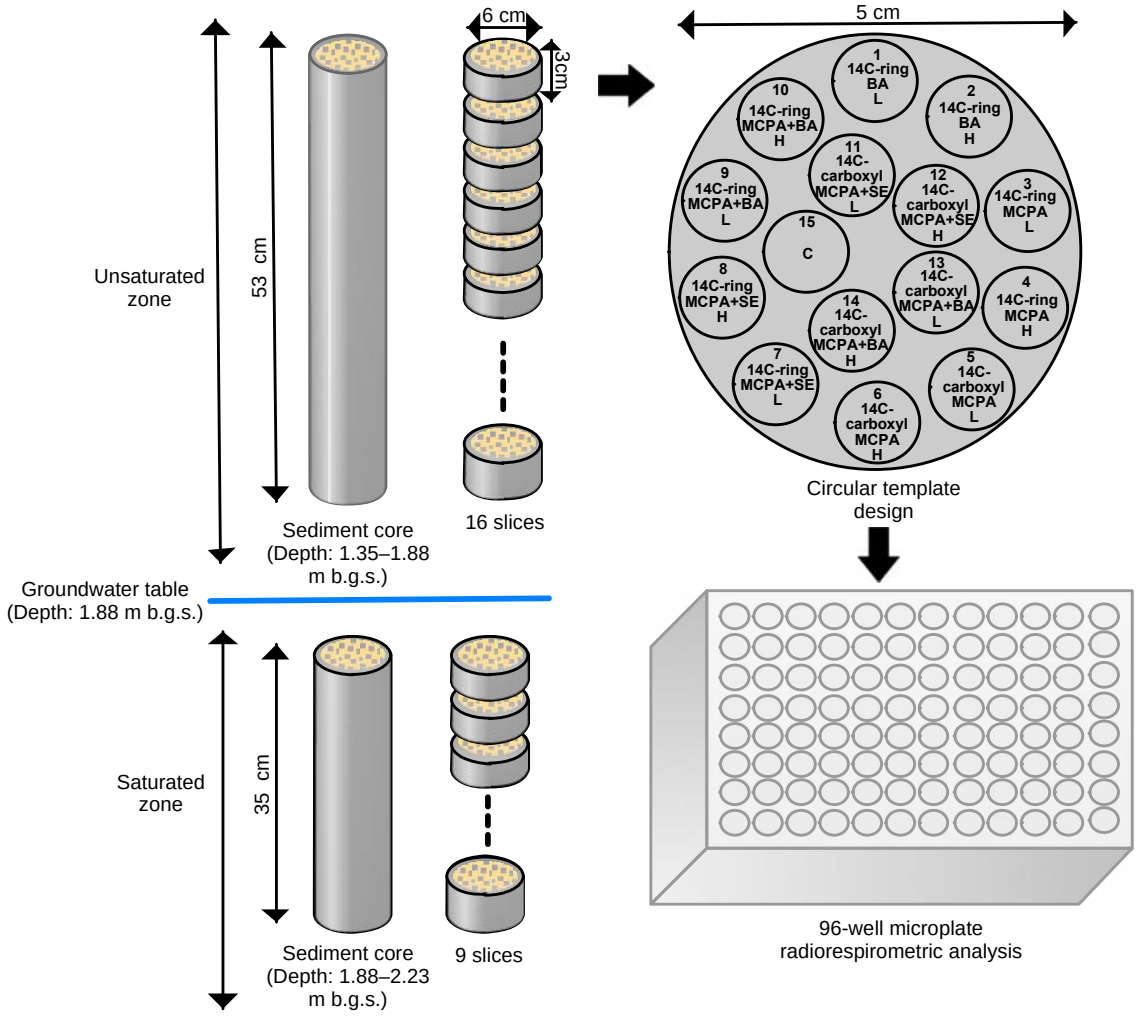

Figure 3. Schematic illustration of centimetre-scale sampling strategy developed for Batıoğlu-Pazarbaşı *et al.*, II. A circular template with 15 holes where placed on each sediment slice and sediments were sampled from the holes using a two ml tip-cut syringe. ^{14}C-ring-BA: Ring labeled benzoic acid, ^{14}C-ring-MCPA: ring labeled-(4-Chloro-2-methylphenoxy)acetic acid (MCPA), ^{14}C-carboxyl-MCPA: carboxyl labeled MCPA, SE: Soil extract, BA: Unlabeled benzoic acid, C: control, H: high concentration and L: low concentration.

4 PHENOXY ACID HERBICIDES

The phenoxyacetic acids such as 2,4-D and MCPA were introduced as selective herbicides for broadleaf weed control in the 1940s. However, the chlorinated phenoxypropionic acids such as MCPP and 2,4-DP were found to be more effective against some weed varieties and used together with phenoxyacetic acids in the 1950s and 60s as stated in Harrison *et al.*, 1998. Phenoxy acids are frequently found in groundwater and surface water; and the contamination can originate from either non-point (diffuse) or point sources as described the sections below:

4.1 CONTAMINATION SOURCE

The contaminants originate from either non-point (diffuse) or point sources and effect microbial communities (Griebler and Luders, 2009). The contamination sources of pesticides, particularly phenoxy acids was mentioned with the examples from literature and the studies of thesis as follows:

4.1.1 NON-POINT (DIFFUSE) SOURCE

Non-point source pollution is caused by rainfall or snowmelt moving over and through the ground. As the runoff moves, it picks up and carries away natural and human-made pollutants, finally depositing them into lakes, rivers, wetlands, coastal waters and groundwaters according to the definition of U.S. Environmental Protection Agency (2010). Pesticide transport processes such as soil surface runoff, interflow, preferential flow, leaching, atmospheric depositions and spray drift lead to nonpoint-source pollution (Müller *et al.*, 2002). In Batıoğlu-Pazarbaşı *et al.*, I and II, the possible leaching of phenoxy acids from overlying agricultural soil through preferential flow paths as diffuse contamination source was attributed to the centimetre-scale vertical variability of mineralization potentials and degrader genes.

4.1.2 POINT SOURCE

Point-source pollution is defined as the discharge of a discrete identifiable source (Léon *et al.*, 2001; Müller *et al.*, 2002). The point sources of contamination to groundwater and surface water systems are septic tanks, fluid storage tanks, landfills, and industrial lagoons (Winter *et al.*, 1998). Inactive landfills are the potential sources of environmental contamination (Kjeldsen *et al.*, 1998; Pérez

Leblic *et al.*, 2012). The leachate migration causes the most significant concern for groundwater pollution (El-Fadel *et al.*, 1997). The landfill leachate as point source contamination was therefore emphasized in our studies addressing the landfill impact on groundwater-surface water transition zones in the thesis (Batıoğlu-Pazarbaşı *et al.*, III and IV).

4.2 Bacterial Degradation

The bacterial metabolism of pesticides can be divided into two categories: 1) metabolism (biodegradation) and 2) cometabolism (fortuitous degradation) (Chung *et al.*, 2000). Metabolism is the degradation of pesticides via bacterial enzymes to produce energy for their growth. In metabolism, a parent compound can be converted to its metabolites (degradation) or inorganic compounds such as CO_2, Cl^- and NH_4 (mineralization) (Hoyle & Ellen, 2000). In cometabolism, compounds are degraded via bacterial enzymes degrading another compound coincidentally. But the bacteria cannot benefit from the cometabolic degradation such as energy production or bacterial growth.

The metabolic degradation of phenoxy acids mostly initiates with the enzymatic conversion of phenoxy acids to phenols and catechols and then proceeds via ortho-cleavage pathway (Häggblom, 1992; Schlömann, 1994). The degradation pathway of 2,4-D in a model microorganism, -*Cupriavidus necator* JMP134- is well known as demonstrated in Figure 4. *Cupriavidus necator* JMP134 degrades 2,4-D by α-ketoglutarate-dependent dioxygenase encoded by *tfdA* gene responsible for the ether bond cleavage to produce 2,4-dichlorophenol (Fukumori & Hausinger, 1993).

4.3 Modelling of Mineralization Kinetics

Modelling is a powerful approach to obtain a comprehensive understanding of the environmental fate of pesticides (Soulas & Lagacherie, 2001). First-order kinetics is the most frequently used biodegradation model to simulate the decrease of residual mass of a chemical compound in a soil system after its application (Dykaar & Kitanidis, 1996). Monod kinetics is used to simulate the increase in the density of microbial cells for the degradation processes dominated by microbial activities (Alexandar, 2004) (Reviewed in Nofziger, 2008). A recent study suggested that first-order degradation models may underestimate risk of pesticide leaching if the pesticide degradation potential needs amplification during degradation (Cheyns *et al.*, 2010). The researchers determined the growth

of an atrazine degrading biomass with the atrazine additions based on Monod kinetics.

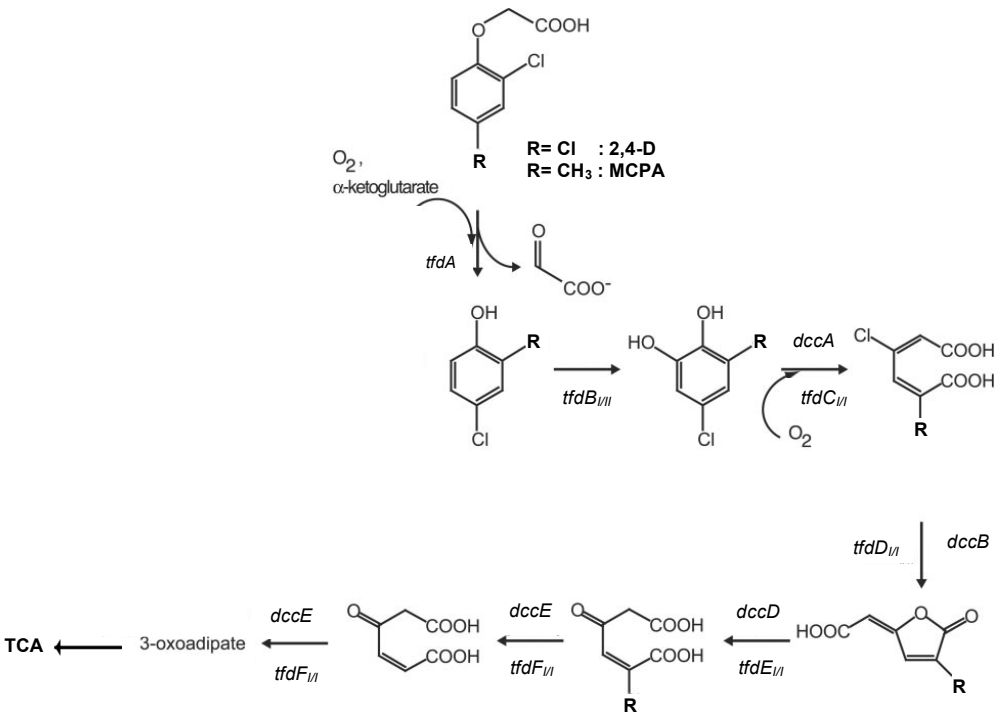

Figure 4. The degradation pathway of 2,4-D and MCPA, and responsible degradative genes in *Cupriavidus necator* JMP134 (Modified from Müller *et al.*, 2004)

Bælum *et al.* (2012) recently used another model called three-half-order kinetics (Brunner & Focht, 1984). This model describes the mineralization kinetics by taking zero-/linear- (Lin.) or exponential (Exp.) growth of the microbial degraders into account. The researchers correlated the high increase in *tfdA* genes to mineralization kinetics taking exponential growth of the degraders into account and the lower *tfdA* content to mineralization kinetics taking linear growth into account. They found that mineralization occurs significantly faster in soils previously treated with phenoxy acid herbicides compared to soils with no exposure history.

The importance of initial biomass expressed as culturable phenoxy acid degraders in the degradation rates was shown in Tuxen *et al.* (2002) with combination of mineralization and Monod kinetics. However, we determined initial biomass by measuring *tfdA* gene classes involved in phenoxy acid degradation; and the differences in the initial abundance of *tfdA* gene classes was correlated with the differences in degradation rates by combining the mineralization and parameterized monod kinetics (Batıoğlu-Pazarbaşı *et al.*, III).

5 BACTERIAL ECOLOGY IN CONTAMINATED ENVIRONMENTS

5.1 SPECIFIC BACTERIAL DEGRADERS

5.1.1 OLIGOTROPHIC BACTERIA

Oligotrophs are characterized both by their inability to grow and prosper in environments with high levels of nutrients, and by an ability to use low concentrations of substrates (Koch, 2001). The absence of light and limited chemical energy resources in non-contaminated groundwater forces the microbial communities to develop specific strategies such as showing small cell sizes, low population densities and reduced activity to survive in oligotrophic conditions (Goldscheider *et al.*, 2006). The oligotroph 2,4-D degrading bacteria such as β proteobacterial *Variovorax* (*tfdA*) and α proteobacterial *Bradyrhizobium* (no *tfdA*) were isolated from different soils with no 2,4-D exposure history (Kamagata *et al.*, 1997). Different catabolic genes from *tfdA* (*cad*-like genes) were determined in oligotrophic *Bradyrhizobium* sp. isolated from a pristine soil (Kitagawa *et al.*, 2002).

In Batıoğlu-Pazarbaşı *et al.*, II, more even centimetre-scale vertical distribution of MCPA mineralization activity at environmentally relevant concentrations than at higher concentrations along the unsaturated- and saturated zones indicated that bacterial communities are capable of degrading low MCPA concentrations more efficiently than higher MCPA concentrations. The presence of oligotrophic bacterial MCPA degraders was attributed to the long-term MCPA exposure at low concentration due to possible MCPA leaching from the overlying agricultural soil into aquifer. In Batıoğlu-Pazarbaşı *et al.*, IV, the relative abundance of oligotrophic flavobacterial family (phylum *Bacteroidetes*) enriched at environmentally relevant concentration rather than higher concentration.

5.1.2 COPIOTROHIC BACTERIA

Copiotrophs are associated with rich environments and generally adapted for using resources rapidly (Koch, 2001). Macur *et al.* (2007) determined that bacterial communities enriched in favor of copiotrophs such as β proteobacterial *Burkholderia* sp. in a 2,4-D-contaminated soil. Similarly, Liu *et al.* (2011) determined that copiotrophic *Sphingomonas*- rather than oligotrophic *Bradyrhizobium*-related taxa consumed the significant amounts of MCPA-derived carbon in the drilosphere and in the soil (depth: 0–2 cm) with their high

nutrient contents. The last study of my thesis also supports that copiotrophs appear after phenoxy acid mineralizations at high concentrations. Specifically, the taxa affiliated to alphaproteobacterial *Sphingomonadaceae* increased during the exponential phases of 2,4-D and 2,4-DP mineralizations at high concentrations rather than environmentally relevant concentrations (Batıoğlu-Pazarbaşı *et al.*, IV).

5.2 Bacterial Adaptation to Long-Term Contamination

Adaptation is defined as the time required to produce a catabolic population or to grow an initially adapted population to a size producing measurable biodegradation (Alexander, 1994). Microorganisms have also multiple mechanisms such as mutation, DNA rearrangement and horizontal gene transfer to adapt to environmental contaminants (van Der Meer *et al.,* 1992). Although the understanding of microbial adaptation to environmental stresses is highly important in ecology and evolution studies, only a limited number of studies recently focused on the responses and the adaptation of microbial communities to long-term contamination in soil (Sheik *et al.*, 2012; Uhlik *et al.*, 2012) and groundwater (Hemme *et al.*, 2010; Batıoğlu-Pazarbaşı *et al.*, IV) by using metagenomic approaches (For more detail, see Section 5.3.2).

In soil, Sheik *et al.* (2012) performed pyrosequencing and determined that long-term exposure to arsenic and chromium highly influenced the diversity and structure of soil microbial communities. In contrast to control soil, *Proteobacteria* dominated in long-term contaminated soils whereas *Actinobacteria* and *Acidobacteria* were the minor components of bacterial community. Uhlik *et al.* (2012) combined stable isotope probing with pyrosequencing and identified dominant proteobacterial genera involved in the degradation of biphenyl, benzoate, and naphthalene in a long-term (~30 year) contaminated soil.

In groundwater, Hemme *et al*. (2010) performed random shotgun sequencing and determined that long-term metal contamination (~50 year) caused a decrease in the diversity dominated by γ- and β-proteobacterial populations (For more detail, see Section 5.3.2). I studied the long-term contamination effect of landfill-derived phenoxy acid herbicides on the microbial diversity of groundwater-surface water transition zones by using pyrosequencing (Batıoğlu-Pazarbaşı *et al.*, IV). The principal coordinate analysis of bacterial pyrosequencing data

disclosed a clustering due to the discharge zones receiving different herbicide mass discharges.

All of these studies clearly explain that long-term contamination has a remarkable impact on the microbial diversity of soil and groundwater systems.

5.3 Molecular Tools in Intrinsic Bioremediation

Bioremediation strategies should be designed based on the presence of microorganisms and, their metabolic capabilities and responses to environmental changes (Lovley, 2003). Molecular tools now allow for the rapid profiling of the microbial communities in pristine and contaminated terrestrial and aquatic environments. Genomic- and metagenomic-based methods were demonstrated in Figure 5; and their applications were given in the following sections:

5.3.1 Genomics in Intrinsic Bioremediation

Real-time quantitative PCR (qPCR) has been used to quantify the abundance of specific microbial groups in environmental samples by enumerating 16S rRNA or functional gene copy numbers. This technique is based on either 5'-nuclease chemistry (e.g., TaqMan assay) or the incorporation of a DNA-binding fluorescent dye (e.g., SYBR Green I) (Drenovsky *et al.,* 2008). The examples from the literature and the qPCR method applied in the thesis were given below:

SYBR green binds to all double-stranded DNA via intercalation between adjacent base pairs, and the fluorescent signals are emitted following light excitation. The melting curve analysis is also carried out to confirm that the fluorescence signal is generated only from target templates and not from the formation of nonspecific PCR products. SYBR Green based qPCR assay was used to quantify *tfdA* genes (Bælum *et al.,* 2006; Gonod *et al.,* 2006b). This assay was also applied to quantify 16S rRNA and *tfdA* gene copy numbers in the DNA samples extracted from our aquifer sediments (Batıoğlu-Pazarbaşı *et al.,* I, II) and streambed sediments (Batıoğlu-Pazarbaşı *et al.,* III and IV).

The TaqMan probes are fluorescently labelled at their 5' ends and contain quencher molecules at their 3' ends (Livak *et al.,* 1995). TaqMan probes can be labelled with different fluorophores to develop multiplex qPCR protocols; and thus different targets can be coamplified and quantified within a single reaction. For instance, Baldwin *et al.* (2003) developed a multiplex qPCR assay targeting a

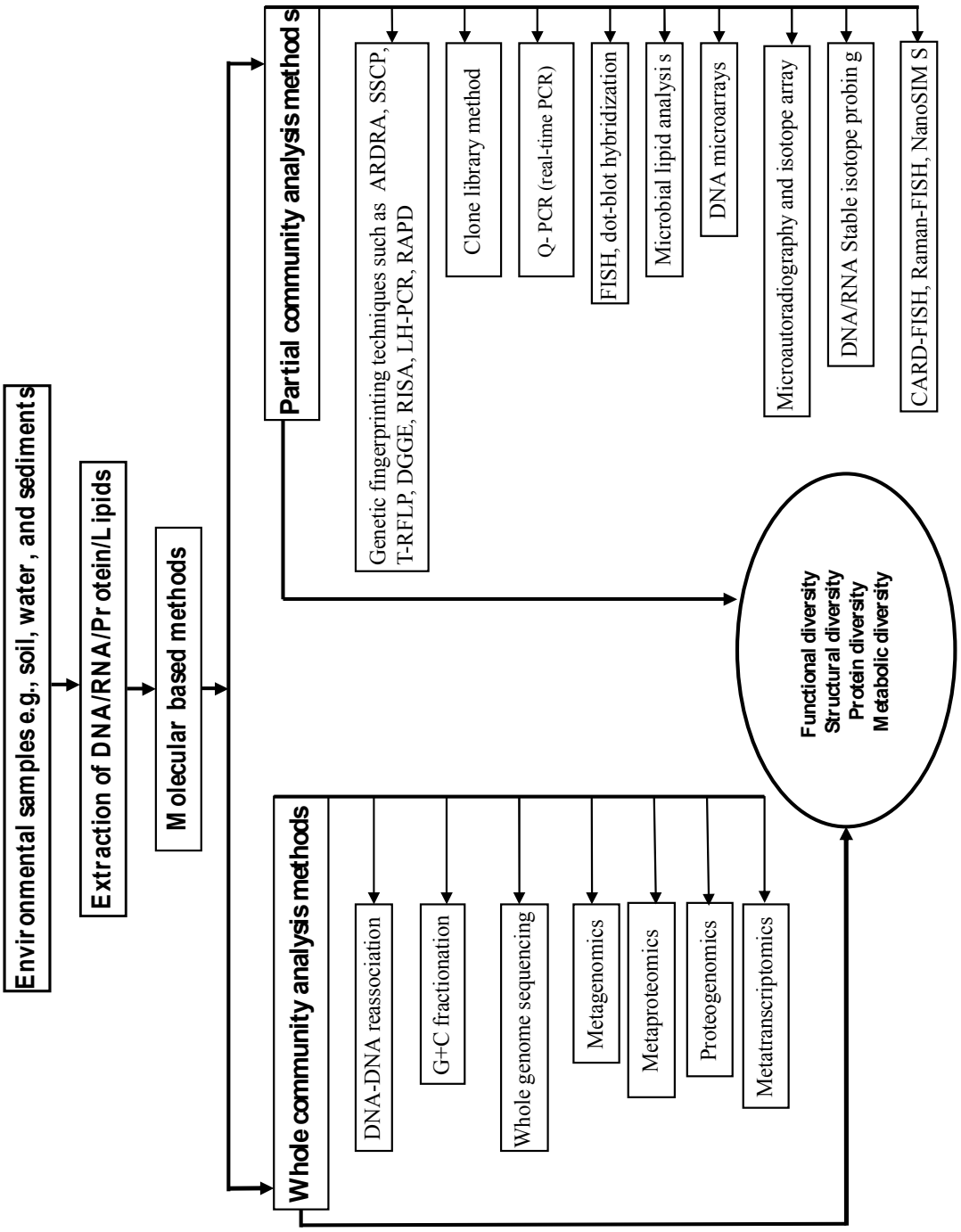

Figure 5. Culture-independent molecular toolbox to characterize the structural and functional diversity of microorganisms in the environment (Reprinted by permission from Springer Science+Business Media: Microbes and Microbial Technology: Agricultural and Environmental Applications (Rastogi G & Sani RK, 2011, Figure 2.1).

number of different aromatic oxygenase genes using bacterial strains and then subsequently applied the assay to simultaneously quantify aromatic oxygenase genes in contaminated groundwater (Baldwin *et al.*, 2008) (Reviewed in Smith & Osborn, 2008).

The steps of TaqMan probe-based triplex qPCR assay used in this thesis were illustrated in Figure 6. This method was previously developed to quantify the three classes of *tfdA* gene involved in phenoxy acid degradation in soil samples by Bælum & Jacobsen (2009). The assay was also applied to simultaneously quantify *tfdA* gene classes in the DNA samples extracted from our aquifer sediments (Batıoğlu-Pazarbaşı *et al.*, I, II) and streambed sediments (Batıoğlu-Pazarbaşı *et al.*, III and IV).

Terminal Restriction Fragment Length Polymorphism (T-RFLP) method was developed for the rapid analysis of microbial diversity in various environments (Liu *et al.*, 1997). In the PCR step of this technique, one primer was fluorescently labeled at the 5′ end, and used to amplify a selected region of bacterial genes encoding 16S rRNA from total community DNA. The PCR product was digested with restriction enzymes; and the fluorescently labeled terminal restriction fragments (T-RF) were precisely measured by using an automated DNA sequencer.

T-RFLP has previously been used to determine the bioremediation effect on the bacterial diversity of a pesticide-contaminated site (Paul *et al.*, 2006), the effect of diuron biotransformation on bacterial biofilm diversity (Vercraene-Eairmal *et al.*, 2010) and the effect of earthworms on MCPA-degrading taxa (Liu *et al.*, 2011). The shift in microbial diversity was also determined with the combination of T-RFLP and 16S rRNA clone library in landfill leachate-contaminated soils in response to phenanthrene amendment (Zhang *et al.*, 2011). Pearce *et al.* (2011) presented a systematic, nonparametric decision-making methodology to help characterizing a water quality gradient in leachate-contaminated groundwater using only microbiological data for input. The data-driven methodology is based on the clustering a set of T-RFLP profiles of groundwater contaminated with multiple pollutants. The authors concluded that the groupings based on microbial data are consistent with the classifications of water quality according to hydrochemical data. These microbial community profile data and improved decision-making strategy complete the traditional chemical groundwater analyses to describe the spatial zones of groundwater contamination.

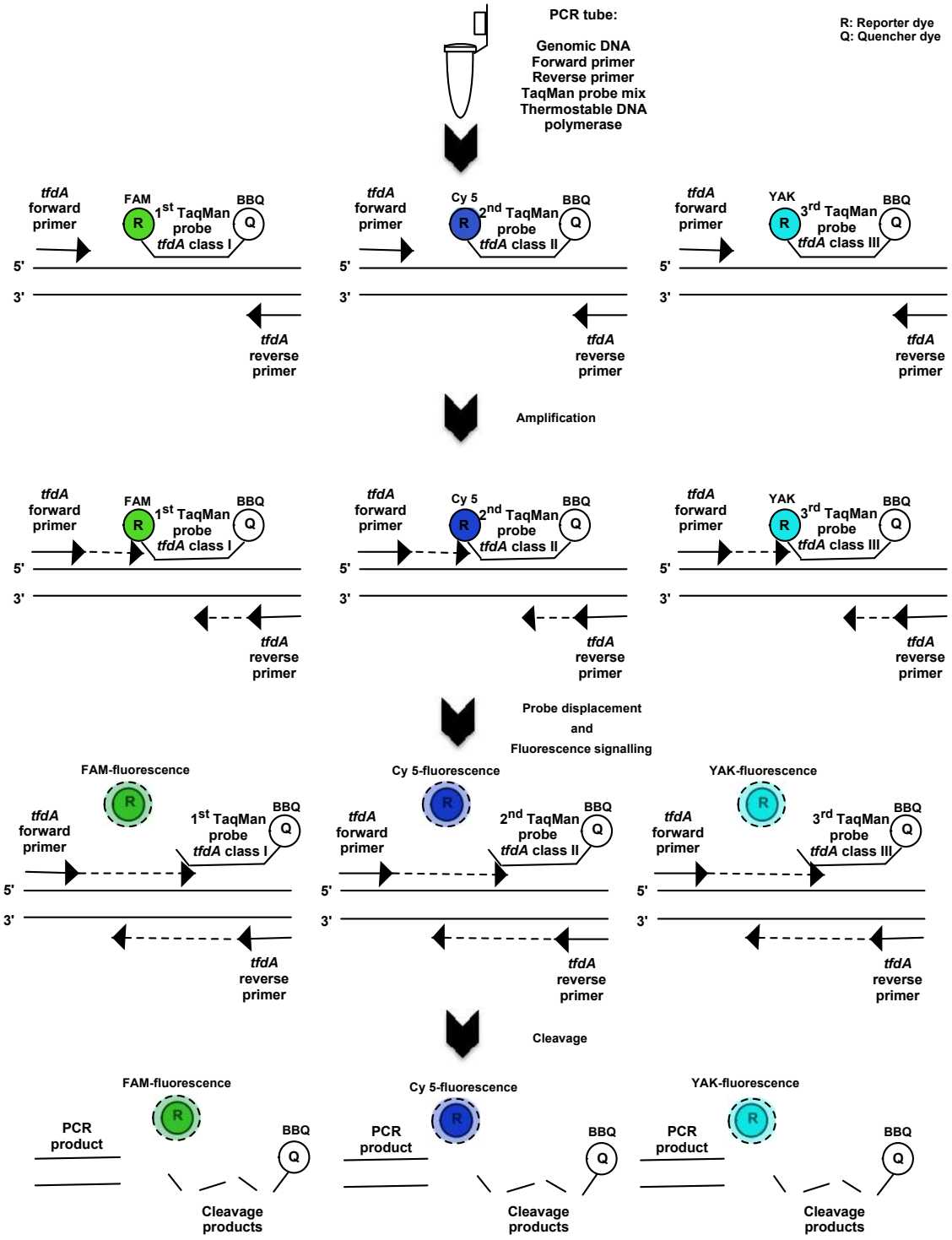

Figure 6. The scheme of TaqMan probe-based real-time triplex qPCR assay used in the studies of this thesis to quantify three classes of *tfdA* gene simultaneously.

In this thesis, the effect of landfill-phenoxy acids on microbial community structure was revealed in streambed sediments along the Risby Stream with the combination of T-RFLP and 16S rRNA amplicon sequencing methods. The microbial community data obtained from both T-RFLP and pyrosequencing were consistently clustered into three groups depending on the long-term exposure of phenoxy acid herbicides leaching from an inactive landfill (Batıoğlu-Pazarbaşı *et al.*, IV). This genomic-metagenomic approach provides microbial ecological indicators to perform more accurate and advanced human health and ecological risk assessments.

5.3.2 METAGENOMICS IN INTRINSIC BIOREMEDIATION

The metagenome was firstly termed for the cloning and functional analysis of the collective genomes of soil microflora (Handelsman *et al.*, 1998). Metagenomics was defined as the genomic analysis of microorganisms by direct extraction and the cloning of DNA from an assemblage of microorganisms (Handelsman, 2004). Metagenomics gives remarkable opportunities to reveal the microbial responses and adaptation to environmental contaminants. However, metagenomic approaches are limited in the studies of intrinsic bioremediation (i.e. natural attenuation).

In Shotgun Sequencing, a large DNA fragment can be completely sequenced by cloning smaller sub-fragments in a single-stranded phage vector to produce a representative library, then sequencing randomly-chosen clones using the dideoxynucleotide chain termination method and a flanking universal primer (Sanger *et al.*, 1977). The final sequence is built up as a composite of overlapping sub-fragment sequences, has been termed as shotgun DNA sequencing (Reviewed in Anderson *et al.*, 1981).

Whole-genome shotgun sequencing is traditionally applied to identify the genome sequence from a particular organism. But Venter *et al.* (2004) used this method to capture a representative sequence from many diverse organisms of the Sargasso Sea. The researchers discovered vast amounts of previously unknown diversity, including over 1.2 million new genes, 148 new species, and numerous new rhodopsin genes with an enormous amount of sequencing (1.6 Gbp) (Reviewed in Chen & Pachter, 2005). However, the first metagenomic analysis addressing the responses and adaptations of groundwater microbial communities to human-induced environmental changes was recently performed (Hemme *et al.*, 2010).

The researchers used random shotgun methods (Tyson *et al.*, 2004) to study the diversity and evolution of a groundwater microbial community in an extreme low-pH environment contaminated with high levels of uranium, nitric acid, technetium and organic solvents. This metagenomic analysis revealed that prolonged exposure to high concentrations of heavy metals, nitric acid and organic solvents (~50 years) has resulted in a massive decrease in species and allelic diversity as well as a significant loss of metabolic diversity. But researchers also discovered that the surviving microbial community has all metabolic pathways necessary for survival and growth in such an extreme environment. More studies are required to understand the bacterial responses and adaptations to a wide range of environmental pollutants to monitor the natural attenuation potentials in groundwater resources.

In High-Throughput Sequencing, two platforms are commonly used for high-throughput sequencing studies: 1) The Roche/454 FLX platform (Margulies *et al.*, 2005) and 2) The Illumina (Solexa) GAIIx platform (Bentley *et al.*, 2006).

In the Roche/454 FLX platform, genomic DNA is isolated, fragmented, ligated to adapters and separated into single strands. Fragments are bound to beads under conditions that favor one fragment per bead, the beads are captured in the droplets of a PCR-reaction-mixture-in-oil emulsion; and PCR amplification occurs within each droplet, resulting in beads each carrying ten million copies of a unique DNA template. The emulsion is broken, the DNA strands are denatured, and beads carrying single-stranded DNA clones are deposited into wells of a fibre-optic slide (picotiter plate). Smaller beads carrying immobilized enzymes required for pyrophosphate sequencing are deposited into each well. The nucleotide incorporation is detected by the associated release of inorganic pyrophosphate and photon generation (Ronaghi *et al.*, 1996, 1998) (Reviewed in Margulies *et al.*, 2005). The picotiter plate is seated opposite a CCD camera that records the light emitted at each bead. The first four nucleotides (TCGA) on the adapter fragment adjacent to the sequencing primer added in library construction correspond to the sequential flow of nucleotides into the flow cell. This strategy allows the 454 base-calling software to calibrate the light emitted by a single nucleotide incorporation (Reviewed in Mardis *et al.*, 2008).

In the Illumina (Solexa) Genome Analyzer platform, sequencing by synthesis is carried out by adding a mixture of four fluorescently labelled reversible chain terminators and DNA polymerase to the template. This results in addition of a single reversible terminator to each template. The fluorescent signal is detected

for each template, and the flourophore and the reversible block are removed. The terminator–enzyme mix is then added to start the next cycle, and the process is reiterated until the end of the run (Reviewed in Bentley *et al.*, 2006).

454 pyrosequencing of 16S rRNA genes has been performed in chromium- and arsenic-contaminated (Sheik *et al.*, 2012), polychlorinated biphenyl- and polyaromatic hydrocarbon-contaminated soils (Uhlik *et al.*, 2012) and polybrominated diphenyl ether contaminated riverside sediment (Xu *et al.*, 2012) to reveal the contaminant effect on microbial community structures. However, 16S rRNA amplicon pyrosequencing approach has not been used to reveal the effect of long-term pesticide exposure on microbial community structures in soil, aquifer sediment or streambed sediments to date. However, the long-term exposure effect of phenoxy acids on microbial communities was studied in the streambed sediments of landfill-impacted groundwater–surface water transition zones as the last study of the thesis (Batıoğlu-Pazarbaşı *et al.*, IV). High-throughput sequencing technologies offer huge opportunities to understand the microbial responses and adaptations to environmental pollutants including pesticides in long-term contaminated subsurface environments prior to any clean up and management of polluted groundwater. The comprehensive microbial surveys can provide useful information in environmental pollution risk assessments and in design of clean-up activities.

5.3.3 Sequence Analysis (Bioinformatics)

The transition from Sanger sequencing to 454 sequencing has opened new horizons in microbial community analysis by making it possible to collect hundreds of thousands of sequences spanning hundreds of samples (Caporaso *et al.*, 2011). The bioinformatic tools are therefore needed for the analysis and comparison of 16S rRNA sequences. The available tools: DOTUR (Schloss & Handelsman, 2005), UniFrac (Lozupone *et al.*, 2006), SINA aligner at the SILVA website (Pruesse *et al.*, 2007), MOTHUR (Schloss *et al.*, 2009), RDP (Cole *et al.*, 2009), EstimateS (Colwell, 2009), MLtreemap (Stark *et al.*, 2010), Pplacer (Matsen *et al.*, 2010), QIIME (Caporaso *et al.*, 2010) and MEGAN (Mitra *et al.*, 2011).

In Batıoğlu-Pazarbaşı *et al.*, IV, QIIME (Quantitative Insights Into Microbial Ecology) toolkit was used to perform the standard methods of microbial community analysis on 16S rRNA sequences, including quality filtering of reads, efficient operational taxonomic unit (OTU) picking, taxonomy assignment,

computation of β diversity measures by taking the relative abundance into account and principal coordinate analysis. The overview of QIIME workflow was given in Figure 7. The modular structure of QIIME allows users to compare environmental samples with the integrated phylogenetic analyses such as UniFrac (Lozupone *et al.*, 2011), and to identify the factors explaining the differences among microbial communities with integrated multivariate statistical tools such as principal coordinate analysis (PCoA). But this advantageous open source pipeline (http://qiime.sourceforge.net) was used in a limited number of studies focusing the contaminant effect on microbial populations (e.g. Kostka *et al.*, 2011 and Ramirez *et al.*, 2012).

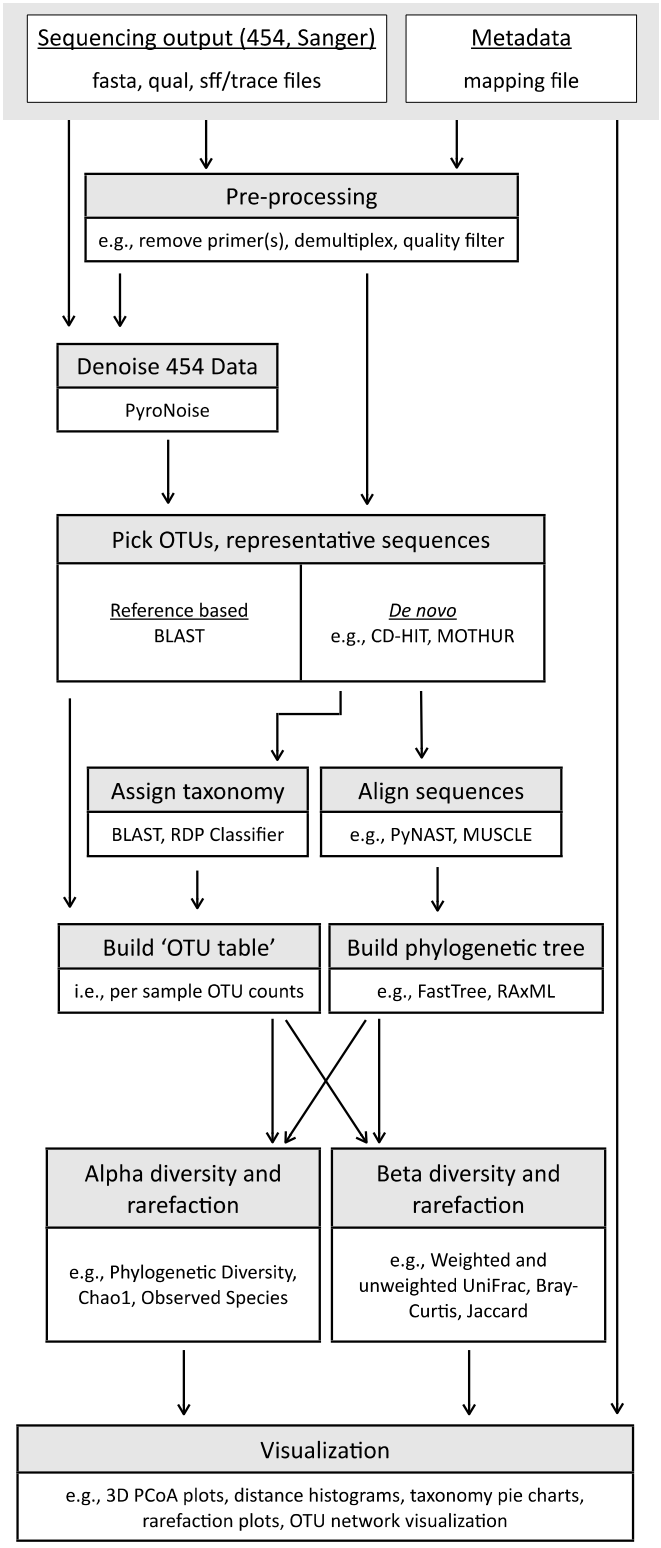

Figure 7. Overview of the QIIME workflow. (Reprinted by permission from Macmillan Publishers Ltd: Nature Methods (Caporaso *et al.*, 2010, Supp. Figure 1).

6 CONCLUSIONS

This PhD thesis focused on the small-scale distribution of phenoxy acid herbicide-mineralization potentials depending on the long-term phenoxy acid herbicide exposure and the bacterial ecology in two important transition zones, (1) the interface of unsaturated- and saturated zones (the aquifer sediment around the groundwater table) and (2) the interface of groundwater and surface water (the streambed sediment).

The major findings of my PhD thesis are summarized in two parts as follows:

1. **The interface of unsaturated- and saturated zones**

- The centimetre-scale vertical variability in 2,4-D and MCPA mineralization potential was found in the aquifer sediment below the groundwater table. The fastest mineralization in the region close to groundwater table and the considerable variability were observed at increasing depth. This vertical variability in 2,4-D and MCPA mineralization was linked to the abundance of total *tfdA*, *tfdA* class I and class III genes and total organic carbon.
- Higher mineralization potential of MCPA than that of 2,4-D in the saturated zone was attributed to the presence of microbial degraders that have adapted to continuous exposure due to MCPA leaching from overlying agricultural soil. The vertical variability in mineralization potential was also correlated with the organic content of the sediment at centimetre scale.
- Higher MCPA mineralization potential was observed at environmentally relevant concentration than at higher concentration along both unsaturated- and saturated zone indicated the presence of bacterial community capable of degrading MCPA at low concentration. Furthermore, the additional carbon sources caused cometabolic MCPA mineralization depending on the abundance of *tfdA* gene classes.

2. The interface of groundwater and surface water

- The phenoxy acids at environmentally relevant concentrations were mineralized to different extents in the streambed sediments according to the changing discharge rates of groundwater contaminated with landfill-phenoxy acids depending on the initial *tfdA* gene classes. Furthermore, the simulated mineralization rates also varied according to discharge zones and the pesticide fate models fitted the observed mineralization curves well and confirmed the importance of initial abundance of *tfdA* gene classes in mineralization kinetics.
- The phenoxy acids at higher concentrations were also mineralized to different extents in the same streambed sediments. According to the results of T-RFLP and pyrosequencing, the specific bacterial communities developed in response to the changing discharge rates of landfill-derived phenoxy acids. Furthermore, the bacterial responses in each discharge zone differentiated followed by mineralization depending on the concentration of herbicides added to microcosms.
- In detail, the highest relative abundance of betaproteobacterial *Comamonadaceae* and the lowest relative abundance of *Acidobacteria Gp 6* were observed in the second discharge zone receiving groundwater at higher pH level when compared to first and third discharge zones. Furthermore, the taxa affiliated to copiotrophic alphaproteobacterial *Sphingomonadaceae* increased temporarily in the first discharge zone only during the exponential phases of 2,4-D and 2,4-DP mineralizations at high concentration. Conversely, the relative abundance of oligotrophic flavobacterial family (phylum *Bacteroidetes*) enriched only in SM2 and only at environmentally relevant concentration.

7 FUTURE DIRECTIONS

Understanding of the biodegradation potential of contaminants plays an important role in the risk assessment of environment and human health for clean-up activities. However, microbial responses to long-term individual or multiple contaminants from diffuse or point sources should also be understood in detail with advanced sequencing technologies and bioinformatic tools. The multidisciplinary efforts are required to incorporate the microbial data into hydrogeological and geochemical analysis.

Environmentally relevant concentrations should be used in mineralization experiments to determine the realistic biodegradation capabilities of microbial populations in soil or subsurface environments. Furthermore, the contaminant effect on microbial community structures in oligotrophic environments should be studied in detail to reveal the microbial communities adapted to the contaminants at low concentrations.

The landfill leachates are highly important source of multiple contaminants. However, the landfill impacts on microbial communities have so far been ignored by microbiologists due to multidisciplinary challenges. In the last two studies of my thesis, I attempted to establish a bridge between hydrogeological (e.g. discharge measurement of landfill-impacted groundwater), geochemical (e.g. phenoxy acid herbicide analysis) and microbiological (e.g. qPCR, T-RFLP, pyrosequencing) aspects. The microbial diversity was screened before, during and after degradation experiments to understand the microbial dynamics. More such approaches can be developed in the future to create hydrogeochemical and molecular toolboxes to clean up contaminated sites.

The phenomenon of spatial variability should also be taken into account with all hydrogeochemical and microbiological aspects in the step of sampling design. Finally, intrinsic bioremediation (i.e. natural attenuation) can be a very powerful strategy in clean-up activities. But the long-term monitoring of natural attenuation should be performed with multifunctional toolboxes including the tools of engineering, genomics/metagenomics, bioinformatics and multivariate statistics.

8 REFERENCES

Affek HP, Ronen D & Yakir, D (1998) About production of CO2 in the capillary fringe of a deep phreatic aquifer. *Water Resour Res* **34:** 989–996.

Alexander, M., 1994. Biodegradation and bioremediation. Academic Press, A division of Harcourt Brace & Company, New York.

Anderson S (1981) Shotgun DNA sequencing using cloned DNase I-generated fragments. *Nucleic Acid Res* **9:** 3015–3027.

Baldwin BR, Nakatsu CH & Nies L (2003) Detection and enumeration of aromatic oxygenase genes by multiplex and real-time PCR. *Appl Environ Microbiol* **69:** 3350–3358.

Baldwin BR, Nakatsu CH & Nies L (2008) Enumeration of aromatic oxygenase genes to evaluate monitored natural attenuation at gasoline-contaminated sites. *Water Res* **42:** 723–731.

Becker MW, Georgian T, Ambrose H, Siniscalchi J & Fredrick K (2004) Estimating flow and flux of ground water discharge using temperature and velocity. *J Hydrol* **296:** 221-233.

Bending G, Shaw E & Walker A (2001) Spatial heterogeneity in the metabolism and dynamics of isoproturon degrading microbial communities in soil. *Biol Fertil Soil* **33:** 484–489.

Bentley DR. (2006) Whole-genome resequencing. *Curr Opin Genet Dev* **16:** 545–52.

Berkowitz B, Silliman SE & Dunn AM (2004) Impact of the Capillary Fringe on Local Flow, Chemical Migration, and Microbiology. *Vad Zone J* **3:** 534–548.

Brad T, van Breukelen BM, Braster M, van Straalen NM & Rölling WFM (2008) Spatial heterogeneity in sediment-associated bacterial and eukaryotic communities in a landfill leachate-contaminated aquifer. *FEMS Microbiol Ecol* **65:** 534–543.

Brunner W & Focht DD (1984) Deterministic 3-half-order kinetic-model for microbial-degradation of added carbon substrates in soil. *Appl Environ Microbiol* **47:** 167–172.

Bundt M, Widmer F, Pesaro M, Zeyer J & Blaser P (2001) Preferential fow paths: biological 'hot spots' in soils. *Soil Biol Biochem* **33:** 729–738.

Bælum J & Jacobsen CS (2009) TaqMan probe-based real-time PCR assay for detection and discrimination of class I, II, and III *tfdA g*enes in soils treated with phenoxy acid herbicides. *Appl Environ Microbiol* **75:** 2969–2972.

Bælum J, Henriksen T, Hansen HCB & Jacobsen CS (2006) Degradation of 4-chloro-2-methylphenoxyacetic acid in top- and sub-soil is quantitatively linked to the class III *tfdA* gene. *Appl Environ Microbiol* **72:** 1476–1486.

Bælum J, Prestat E, David MM, Strobel BW & Jacobsen CS (2012) Modelling phenoxy acid herbicide mineralization and growth of microbial degraders in 15 soils monitored by quantitative real-time PCR of the functional *tfdA* gene. *Appl Environ Microbiol* doi:10.1128/AEM.00990-12.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7:** 335-336.

Caporaso JG, Lauber CL, Walters WA, Berg-lyons D, Lozupone CA *et al.* (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *PNAS* **108:** 4516–4522.

Chen K & Pachter L (2005) Bioinformatics for whole-genome shotgun sequencing of microbial communities. *PLoS Comp Biol* **1:** e24.

Chung K-Y (2000) Enhanced degradation of pesticides. *Environ Eng Res* **5:** 47–61.

Cole JR, Wang Q, Cardenas E, Fish J, Chai B *et al.* (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucl Acids Res* **37:** D141-D145.

Colwell RK (2009) EstimateS: Statistical estimation of species richness and shared species from samples. Version 8.2. User's Guide and application, published at: http://purl.oclc.org/estimates.

Conant B Jnr (2004) Delineating and quantifying ground water discharge zones using streambed temperatures. *Ground Water* **42:** 243-257.

Cubillos-Ruiz A, Junca, H, Baena S, Venegas, I & Zambrano MM (2010) Beyond Metagenomics: Integration of Complementary Approaches for the Study of Microbial Communities. *Metagenomics: Theory, Methods and Applications* (Marco D, ed), pp. 15–37. Caister Academic Press.

Dobson R, Schroth MH & Zeyer J (2007) Effect of water-table fluctuation on dissolution and biodegradation of a multi-component, light nonaqueous-phase liquid. *J Contam Hydrol* 94: 235–248.

Dykaar BB & Kitanidis PK (1996) Macrotransport of a biologically reacting solute through porous media. *Water Resour Res* **32:** 307–320.

El-Fadel M, Findikasis AN & Leckie JO (1997) Modelling leachate generation and transport in solid waste landfills. *Environ Technol* 18: 669–686.

El Sebai T, Lagacherie B, Soulas G & Martin-Laurent F (2007) Spatial variability of isoproturon mineralizing activity within an agricultural field: geostatistical analysis of simple physicochemical and microbiological soil parameters. *Environ Pollut* **145:** 680–690.

Fisher SG, Grimm NB, Marti E, Holmes RM & Jones JB (1998) Material spiraling in stream corridors: A telescoping ecosystem model. *Ecosystems* **1:** 19–34.

Franklin RB & Mills AL (2003) Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. *FEMS Microbiol Ecol* **44:** 335–346.

Franklin RB & Mills AL (2007) The importance of microbial distribution in space and spatial scale to microbial ecology. *The Spatial Distribution of Microbes in the Environment* (Franklin RB & Mills AL, eds), pp. 1–30. Springer.

Ford RG, Wilkin RT & Hernandez G (2006) Arsenic cycling within the water column of a small lake receiving contaminated groundwater discharge. *Chem Geol* **228:** 137–155.

Fukumori F & Hausinger RP (1993) Purification and characterization of 2,4-dichlorophenoxyacetate/α-ketoglutarate dioxygenase. *J Biol Chem* **268:** 24311–24317.

Gintautas PA, Daniel SR & Macalady DL (1992) Phenoxyalkanoic Acid Herbicides in Municipal Landfill Leachates. *Environ Sci Technol* **26:** 517–521.

Goldscheider N, Hunkeler D & Rossi P (2006) Review: Microbial biocenoses in pristine aquifers and an assessment of investigative methods. *Hydrogeol J* **14:** 926–941.

Gonod LV, Chenu C & Soulas G (2003) Spatial variability of 2,4-Dichlorophenoxy-acetic acid (2,4-D) mineralization potential at a millimetre scale in soil. *Soil Biol Biochem* **35:** 373–382.

Gonod LV, Chadoeuf J & Chenu C (2006a) Spatial distribution of microbial 2,4-dichlorophenoxy acetic acid mineralization from field to microhabitat scales. *Soil Sci Soc Am J* **70:** 64–71.

Gonod LV, Martin-Laurent F & Chenu C (2006b) 2,4-D impact on bacterial communities, and the activity and genetic potential of 2,4-D degrading communities in soil. *FEMS Microbiol Ecol* **58:** 529–537.

Griebler C & Lueders T (2009) Microbial biodiversity in groundwater ecosystems. *Freshwat Biol* **54:** 649–677.

Haarstad K & Maehlum T (2008) Pesticides in Norwegian Landfill Leachates. *The Open Environmen Biol Monit J* **1:** 8–15.

Handelsman J, Rondon MR, Brady SF, Clardy J & Goodman RM (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chem & Biol* **5:** R245–249.

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. *Microbiol Mol Biol Rev* **68:** 669–685.

Harrison I, Leader RU, Higgo JJ & Williams GM (1998) A study of the degradation of phenoxyacid herbicides at different sites in a limestone aquifer. *Chemosphere* **36:** 1211–1232.

Hemme CL, Deng Y, Gentry TJ, Fields MW, Wu L *et al.* (2010) Metagenomic insights into evolution of a heavy metal-contaminated groundwater microbial community. *The ISME J* **4:** 660–672.

Hoyle BL & Arthur EL (2000) Biotransformation of pesticides in saturated-zone materials. Hydrogeol J **8:** 89–103.

Häggblom MM (1992) Microbial breakdown of halogenated aromatic pes- ticides and related compounds. *FEMS Microbiol Rev* **103:** 29–72.

Jacobsen OS, Brüsch W, Fomsgaard IS, Helweg A, Vinther FP, Spliid NH, Bossi R & Mogensen BB (1998) *Leaching and Degradation of Selected Pesticides in a Sandy Watershed.* Ministry of Environment and Energy, Danish EPA, Copenhagen, Denmark.

Janniche GS, Mouvet C & Albrechtsen H-J (2011) Vertical small scale variations of sorption and mineralization of three herbicides in subsurface limestone and sandy aquifer. *J Contam Hydrol* **123:** 167–177.

Kamagata Y, Fulthorpe RR, Tamura K, Takami H, Forney LJ & Tiedje JM (1997) Pristine environments harbor a new group of oligotrophic 2,4-dichlorophenoxyacetic acid-degrading bacteria. *App Environ Microbiol* **63:** 2266–2272.

Kim, Y-J, Darnault CJG, Bailey NO, Parlange JY & Steenhuis TS (2005) Equation for Describing Solute Transport in Field Soils with Preferential Flow Paths. *Soil Sci Soc Am* **69:** 291–300.

Kitagawa W, Takami S, Miyauchi K, Masai E, Kamagata Y *et al.* (2002) Novel 2,4-Dichlorophenoxyacetic Acid Degradation Genes from Oligotrophic Bradyrhizobium sp . Strain HW13 isolated from a pristine environment. *J Bacteriol* **184:** 509–518.

Kjeldsen P, Grundtvig AA, Winther P & Andersen JA (1998) Characterization of an old municipal landfill (Grindsted, Denmark) as a groundwater pollution source: landfill history and leachate composition. *Waste Manag Res* **16:** 3–13.

Koch L (2001) Oligotrophs versus copiotrophs. *BioEssays* **23:** 657–661.

Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G *et al.* (2011) Hydrocarbon-degrading bacteria and the bacterial community response in gulf of Mexico beach sands impacted by the deepwater horizon oil spill. *Appl Environmen Microbiol* **77:** 7962–7974.

Larsen L, Sørensen SR & Aamand J (2000) Mecoprop, Isoproturon, and Atrazine in and above a Sandy Aquifer : Vertical Distribution of Mineralization Potential. *Environ Sci Technol* **34:** 2426–2430.

Lehman RM (2007) Understanding of aquifer microbiology is tightly linked to sampling approaches. *Geomicrobiology* **24:** 331–341.

Léon LF, Soulis ED, Kouwen N & Farquhar GJ (2001) Non- point source pollution: A distributed water quality modelling approach. *Water Res* **35:** 997–1007.

Livak KJ, Flood SJ, Marmaro J, Giusti W & Deetz K (1995) Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl* **4:** 357–362.

Liu W-T, Marsh TL & Cheng H & Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environmen Microbiol* **63:** 4516–4522.

Liu Y-J, Liu S-J, Drake HL & Horn M (2011) Alphaproteobacteria dominate active 2-methyl-4-chlorophenoxyacetic acid herbicide degraders in agricultural soil and drilosphere. *Environmen Microbiol* **13:** 991–1009.

Lovley DR (2003) Cleaning up with genomics: applying molecular biology to bioremediation. *Nature Rev* **1:** 35–44.

Lozupone C, Hamady M & Knight R (2006) UniFrac - an online tool for comparing microbial community diversity in a phylogenetic context. *BMC Bioinformatics* **7:** 371.

Lozupone C, Lladser ME, Knights D, Stombaugh J & Knight R (2011) UniFrac: an effective distance metric for microbial community comparison. *ISME J* 5: 169–172.

Macur RE, Wheeler JT, Burr MD & Inskeep WP (2007) Impacts of 2,4-D application on soil microbial community structure and on populations associated with 2,4-D degradation. *Microbiol Res* **162:** 37–45.

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, *et al.* (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437:** 376–80.

Mardis ER (2008) Next-generation DNA sequencing methods. *Annu Rev Genomics Hum Genet* **9:** 387–402.

Matsen FA, Kodner RB & Armbrust EV (2010) Pplacer: linear time maximum-likelihood and Bayesian phylogenetic placement of sequences onto a fixed reference tree. *BMC Bioinformatics* **11:** 538+.

Mitra S, Stärk M & Huson DH (2011) Analysis of 16S rRNA environmental sequences using MEGAN. *BMC genomics* **12:** S17.

Müller K, Bach M, Hartmann H, Spiteller M, Frede H-G (2002) Point- and nonpoint-source pesticide contamination in the Zwester Ohm catchment, Germany. *J Environmen Qual* **31:** 309–318.

Müller TA, Byrde SM, Werlen C, Meer JR & Kohler H-PE (2004) Genetic analysis of phenoxyalkanoic acid degradation in *Sphingomonas herbicidovorans* MH. *Appl Environmen Microbiol* **70:** 6066–6075.

Nofziger DL (2008) First-order degradation of pesticides: Theory http://soilphysics.okstate.edu/software/FirstOrderDegradation.

Paul D, Pandey G, Meier C, van der Meer JR & Jain RK (2006) Bacterial community structure of a pesticide-contaminated site and assessment of changes induced in community structure during bioremediation. *FEMS Microbiol Ecol* **57:** 116–127.

Pearce AR, Rizzo DM & Mouser PJ (2011) Subsurface characterization of groundwater contaminated by landfill leachate using microbial community profile data and a nonparametric decision-making process. *Wat Resour Res* **47:** 1–11.

Pérez-Leblic MI, Turmero A, Hernández M, Hernández AJ, Pastor J, Ball AS, Rodríguez J & Arias ME (2012) Influence of xenobiotic contaminants on landfill soil microbial activity and diversity. *J Environmen Manag* **95:** 285–290.

Pruesse E, Quast C, Knittel K, Fuchs B, Ludwig W *et al.* (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucl Acids Res* **35:** 7188–7196.

Ramirez KS, Craine JM & Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Glob Chan Biol* **18:** 1918–1927.

Rasmussen J, Aamand J, Rosenberg P, Jacobsen OS & Sørensen SR (2005) Spatial variability in the mineralization of the phenylurea herbicide linuron within a Danish agricultural field: multivariate correlation to simple soil parameters. *Pest Man Sci* **61:** 829–837.

Rastogi G & Sani RK (2011) Molecular Techniques to Assess Microbial Community Structure, Function, and Dynamics in the Environment. *Microbes and Microbial Technology: Agricultural and Environmental Applications* (Ahmad I, Ahmad, F & Pichtel J, eds), pp. 29–57. Springer.

Rodriguez-Cruz MS, Bælum J, Shawc LJ, Sørensen SR, Shi S, Aspray T, Jacobsen CS & Bending GD (2009) Biodegradation of the herbicide mecoprop-p with soil depth and its relationship with class III *tfdA* genes. *Soil Biol Biochem* **42:**32–39.

Ronaghi M, Karamohamed S, Pettersson B, Uhlén M & Nyrén P (1996) Real-time DNA sequencing using detection of pyrophosphate release. *Anal Biochem* **242:** 84–89.

Ronaghi M, Uhlén M & Nyrén P (1998) A sequencing method based on real-time pyrophosphate. *Science* **281:** 363–-365.

Ronen D, Scher, H & Blunt, M (1997) On the structure and flow processes in the capillary fringe of phreatic aquifers. *Transp Porous Media* **28:**159–180.

Ronen D, Scher H & Blunt, M (2000) Field observations of a capillary fringe before and after a rainy season. *J Contam Hydrol* **44:** 103–118.

Röling WFM, van Breukelen BM, Braster M, Lin B & van Verseveld HW (2001) Relationships between microbial community structure and hydrochemistry in a landfill leachate-polluted aquifer. *Appl Environmen Microbiol* **67:** 4619–4629.

Sanger F, Nicklen S & Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc Nati Acad Sci* **74:** 5463–5467.

Schloss PD & Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* **71:**1501–1506.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M *et al.* (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl Environ Microbiol* **75:** 7537–7541.

Schlömann M (1994) Evolution of chlorocatechol catabolic pathways. Conclusions to be drawn from comparisons of lactone hydrolases. *Biodegrada- tion* **5:** 301–33.

Sheik CS, Mitchell TW, Rizvi FZ, Rehman Y, Faisal M *et al.* (2012) Exposure of soil microbial communities to chromium and arsenic alters their diversity and structure. *PloS one* **7:** e40059.

Silliman SE, Berkowitz B, Simunek J & van Genuchten MT (2002) Fluid flow and solute migration within the capillary fringe. *Ground Water* **40:** 76–84.

Sinke AJC, Dury O & Zobrist J (1998) Effects of a fluctuating water table: column study on redox dynamics and fate of some organic pollutants. *J Contam Hydrol* **33:** 231–246.

Sjøholm OR, Aamand J, Sørensen J & Nybroe O (2010) Degrader density determines spatial variability of 2,6-dichlorobenzamide mineralization in soil. *Environ Pollut* **158:** 292–298.

Smith, J.W.N., Lerner, D.N., 2008. Geomorphologic control on pollutant retardation at the groundwater–surface water interface. *Hydrol Proc* **22:** 4679–4694.

Smith JWN, Surridge BWJ, Haxton TH & Lerner DN (2009) Pollutant attenuation at the groundwater–surface water interface: A classification scheme and statistical analysis using national-scale nitrate data. *J Hydrol* **369:** 392–402.

Sophocleous M (2002) Interactions between groundwater and surface water: the state of the science. *Hydrogeol J* **10:** 52–67.

Soulas G & Lagacherie B (2001) Modelling of microbial degradation of pesticides in soils. *Biol Fertil Soils* **33:** 551–557.

Stagnitti F, Parlange J-Y, Steenhuis TS, Nijssen B & Lockington D (1994) Modelling the migration of water soluble contaminants through preferred paths in the soils. *Groundwater quality management* (Kovar, K & Soveri, J eds), pp. 367–379. Int Assoc of Hydrological Sci Press.

Stark M, Berger S, Stamatakis A & von Mering C (2010) MLTreeMap - accurate Maximum Likelihood placement of environmental DNA sequences into taxonomic and functional reference phylogenies. *BMC Genomics* **11:** 461+.

Stumpp C & Maloszewski P (2010) Quantification of preferential flow and flow heterogeneities in an unsaturated soil planted with different crops using the environmental isotope δ18O. *J Hydrol* **394:** 407–415.

Stumpp C, Maloszewski P, Stichler W & Maciejewski S (2007) Quantification of the heterogeneity of the unsaturated zone based on environmental deuterium observed in lysimeter experiments. *Hydrol Sci* **52:** 748–762.

Swensen B (1997) Unsaturated flow in a layered, glacial-contact delta deposit measured by the use of 18O, Cl- and Br- as tracers. *Soil Sci* **162:** 242–253.

Tuxen, N, Ejlskov P, Albrechtsen H-J, Reitzel L A, Pedersen JK & Bjerg PL (2003) Application of natural attenuation to ground water contaminated by phenoxy acid herbicides at an old landfill (Sjoelund, Denmark). *Groundwater Monit R* **23:** 48–58.

Tuxen N, Tüchsen PL, Rügge K, Albrechtsen H-J & Bjerg PL (2000): The fate of seven pesticides in an aerobic aquifer studied in column experiments. *Chemosphere* **41:** 1485–1494.

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ *et al.* (2004). Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* **428:** 37–43.

Uhlik O, Wald J, Strejcek M, Musilova L, Jakub Ridl *et al.* (2012) Identification of bacteria utilizing biphenyl, benzoate, and naphthalene in long-term contaminated soil. *PloS one* **7:** e40653.

Van Der Meer JR, De Vos WM, Harayama S & Zehnder AJ (1992) Molecular mechanisms of genetic adaptation to xenobiotic compounds. *Microbiol Mol Biol Rev* **56:** 677–694.

Van Elsas JD & Smalla K (1997) Methods for sampling soil microbes. In: Hurst, C.J., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D., Walter, M.V. (Eds.), Manual of Environmental Microbiology. ASM Press, Washington, DC, pp. 383–390.

Venter JC, Remington K, Heidelberg JF, Halpern AL & Rusch D *et al.* (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304:** 66–74.

Vercraene-Eairmal M, Lauga B, Saint Laurent S, Mazzella N, Boutry S, Simon M, Karama S, Delmas F & Duran R (2010) Diuron biotransformation and its effects on biofilm bacterial community structure. *Chemosphere* **81:** 837–843.

White DS, Elzinga CH & Hendricks SP (1987) Temperature patterns within the hyporheic zone of a northern Michigan river. *J North Am Benthol Soc* **6:** 85–91.

Winter TC, Harvey JW, Franke OL & Alley WM (1998) Ground Water and surface water: A single resource. *US Geological Survey Circular* 1139.

Weiss JV & Cozzarelli IM (2008) Biodegradation in contaminated aquifers: incorporating microbial/molecular methods. *Groundwater* **46:** 305–322.

Xu M, Chen X, Qiu M, Zeng X, Xu J *et al.* (2012) Bar-coded pyrosequencing reveals the responses of PBDE-degrading microbial communities to electron donor amendments. *PloS one* **7:** e30439.

Zhang SY, Wang QF & Xie SG (2011) Microbial community changes in contaminated soils in response to phenanthrene amendment. *Int J Environ Sci Tech* **8:** 321–330.

9 PAPERS

I. Batıoğlu-Pazarbaşı M, Bælum J, Johnsen AR, Sørensen SR, Albrechtsen H-J & Aamand, J (2012) Centimetre-scale vertical variability of phenoxy acid herbicide mineralization potential in aquifer sediment relates to the abundance of *tfdA* genes. *FEMS Microbiology Ecology* **80:** 331–341.

II. Batıoğlu-Pazarbaşı M, Sørensen SR & Aamand, J (2012) Herbicide concentration effect on centimetre-scale spatial distribution of MCPA mineralization potential along the transition zone between unsaturated and saturated zones. Manuscript.

III. Batıoğlu-Pazarbaşı, M, Milosevic, N, Malaguerra, F, Binning, PJ, Albrechtsen, H-J, Bjerg, PL & Aamand, J (2013) Discharge of landfill leachate to streambed sediments impacts the mineralization potential of phenoxy acid herbicides depending on the initial abundance of *tfdA* gene classes. *Environmental Pollution* DOI: 10.1016/j.envpol.2013.01.050.

IV. Batıoğlu-Pazarbaşı M, Bælum J, Pilloni G, Larentis M, Jacobsen CS, Lueders T, Hansen LH & Aamand J (2012) Bacterial adaptive response to changing herbicide discharge rates in the streambed sediments impacted by a landfill. Manuscript.

In this online version of the thesis, the papers are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from:

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The Department of Environmental Engineering (DTU Environment) conducts science-based engineering research within four sections:
Water Resources Engineering, Urban Water Engineering,
Residual Resource Engineering and Environmental Chemistry & Microbiology.

The department dates back to 1865, when Ludvig August Colding, the founder of the department, gave the first lecture on sanitary engineering as response to the cholera epidemics in Copenhagen in the late 1800s.

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